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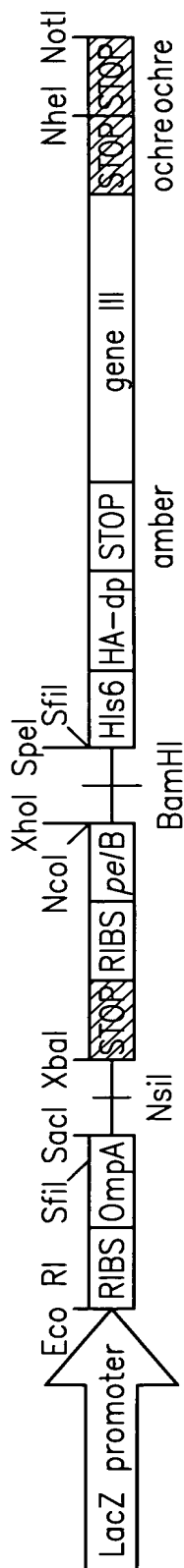


FIG. 1

Human Antibody sequence (TT sequence) (SEQ. ID NO: 54)

Heavy Chain: cloning sites Xho I and Spe I are underlined

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1                               11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21                               31
GTC TCC TGC AGG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG GTG CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41                               51
GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61                               71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG GAC GAA TCC ACG GGC ACA GCC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81                               91
TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GGG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101                              111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121                              131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141                              151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161                              171
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG
glu pro val thr val ser trp asn ser gly ala leu thr ser gly val his thr phe pro
181                              191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201                              211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn thr lys val
221                              231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act agt
asp lys lys val glu pro lys ser cys asp lys thr ser

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FIG. 2A

Human Antibody sequence (TT sequence) (SEQ. ID NO: 55)

Light Chain: cloning sites Sac I and Xba I are underlined

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1                               11
gag ctc acg cag tct cca ggc acc ctg tct ttg tct ccA ggg gaa aga gcc acc ctc tcc
glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser
21                               31
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41                               51
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg
61                               71
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu
81                               91
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acC
asp phe ala val tyr tyr cys gln gln tyr gly gly ser pro trp phe gly gln gly thr
101                              111
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp
121                              131
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg
141                              151
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser
161                              171
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser
181                              191
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser
201                              211
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc tag a
leu pro val thr lys ser phe asn arg gly glu cys AMB

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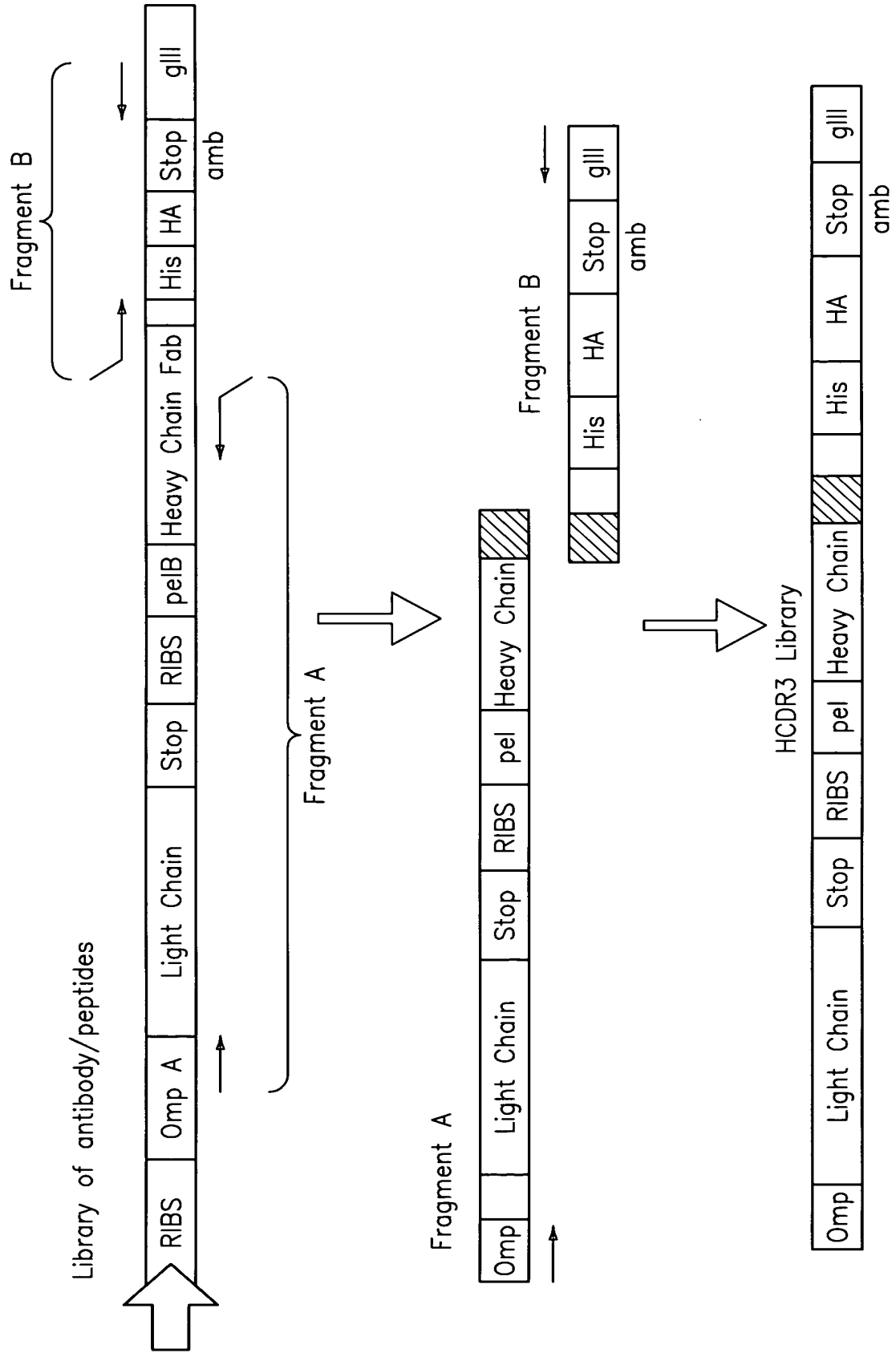
FIG. 2B

Method of grafting peptide into antibody with random sequences
surrounding peptide sequence

	FR3				TPO Mimetic Peptide			
(SEQ ID NO. 56)	Y	Y	C	A	R	X	X	Q
(SEQ ID NO. 57)	TATTAT-TGT-GCG-AGA-NNR-NNR-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-							
	FR4							
	W	L	A	A	R	A	X	T
	TGG-CTG-GCG-GCG-GCG-GCG-NNY-NNY-TGG-GGC-CAA-GGG-ACC-							

The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X"s in the figure.

FIG. 3

**FIG. 4**

CLONE	AMINO ACID SEQUENCE	SEQ ID NO.
X1a	Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-GGA-GGC	25 26
X1a-11	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-GGC-GGA	27 28
X1a-13	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-GGA-GGC	29 30
X1c	Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCT-GTC	31 32
X2c	Mat-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-GTT-GGC	33 34
X3a	Val-Val-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val GTG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCT-GTT	35 36
X3b	Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCC-GAT	37 38
X4b	Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val TTG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCT-GTT	39 40
X4c	Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Ile TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCC-ATC	41 42
X5a	Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCC-GTT	43 44
X5c	Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCT-GTC	45 46
X7a	Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser ACA-CGG-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-TGC-AGC	47 48
X7b	no peptide deletion mutant	
X7c	Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CCG-CAA-TGG-CTG-GCG-GCG-CCT-CAC	49 50

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(SEQ ID NO: 60)

GGGAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAA
ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATC
AAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAACAAGAGT
CCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCCCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTC
GAGGTGCCGTAAAGCACTAAATCGGAACCCCTAAAGGGAGCCCCCGATTAGA
GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGT
AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC
ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGGTGAAA
GTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTG
GTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTT
ACTTCTGACAACGATGGGAGGACCGAAGGAGCTAACCCTTTTTTGCACAAC
ATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAG
CCATAACCAACGACGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAAGG
ATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCG
CTACCAGCGGTGGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACC GCCTACATACCTCGC
TCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA
CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

FIG. 6A

ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
 AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
 ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGT
 TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
 AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTG
 CTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA
 CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACC
 GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAA
 CCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTT
 TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTC
 ACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTG
 TGGAATTGTGAGCGGATAACAATTGAATTCAGGAGGAATTTAAAATGAAAA
 GACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTGGCCAG
 GCGGCCGAGCTCGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCG
 GCTGCCGTAGGCAATAGGTATTTCAATTATGACTGTCTCCTTGGCGACTAGCTA
 GTTTAGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCC
 GCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGG
 GGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGC
 TTTCCAGTCGGGAAACCTGTCGTGTTACTAATGATGGTGATGGTGATGGCTAG
 TTTTGTGACAAGATTTGGGCTCAACTTTCTTGTCCACCTTGGTGTTGCTGGGT
 TGTGATTCACGTTGCAGATGTAGGTCTGGGTGCCCAAGCTGCTGGAGGGCAC
 GGTCACCACGCTGCTGA_gGGAGTAGAGTCCTGAGGACTGTAGGACAGCCGGG
 AAGGTGTGCACGCCGCTGGTCA_gGGCGCCTGA_gTTCCACGACAC_cGTCGCCGG
 TTC_gGGGAAGTAGTCCTTGACCAGGCAGCCAGGGCCGCTGTGCCCCCAGAG
 GTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTGAG
 GCTGCGGAGACGGTGACCGTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTT
 AGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
 CTGAAGATTTTGCAGTGTACTACTGTGTCAGCAGTATGGTGGCTCACCGTGGTTC
 GGCCAAGGGACCAAGGTGGAACCTCAAACGAACTGTGGCTGCACCATCTGTCT
 TCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTG
 TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
 ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAG
 CAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
 CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCacccatcagggcctgagttcgcccgtcac
 aaagagcttcaacggaggagagtgttaatTCTAGATAATTAATTAGGAGGAATTTAAAATGAA
 ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
 CCATGGCCGAGGTGCAGCTGCTCGAGATGAGCGATAAAATTATTCACCTGAC
 TGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTC
 GATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGG
 ATGAAATCGCTGACGAATATCAGGGCAAACCTGACCGTTGCAAACTGAACAT
 CGATCAAAACCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACT
 CTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTTG
 TCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCGTACCCGTACG
 ACGTTCCGGACTACGTTCTACTAGTccgaaaccgtctacccaccgggctcttccctgcggtggccgc
 atcgcccgctcgaggaaaaagtgaaccctgaaagctcagaactccgagctggcgctccactgccaatgctgcgcgaa

FIG. 6B

aggtggcacagctgaaacagaaagttatgaaccatggcggttgtgctagtGGCCAGGCCGGCCAGCACCAT
CACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACCTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCG
ATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGT
GATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTC
ACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCAC
CTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTA
AGCTAGCTAATTAATTTAAGCGGCCGCAGATCT

FIG. 6C

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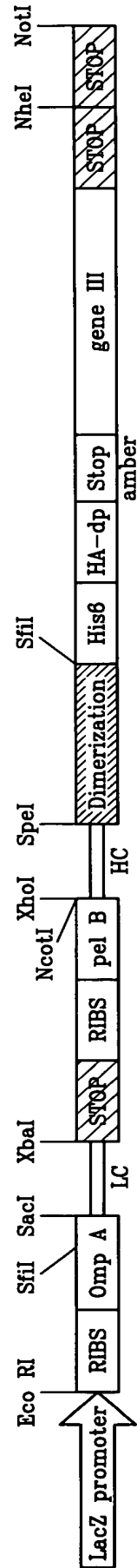


FIG. 7

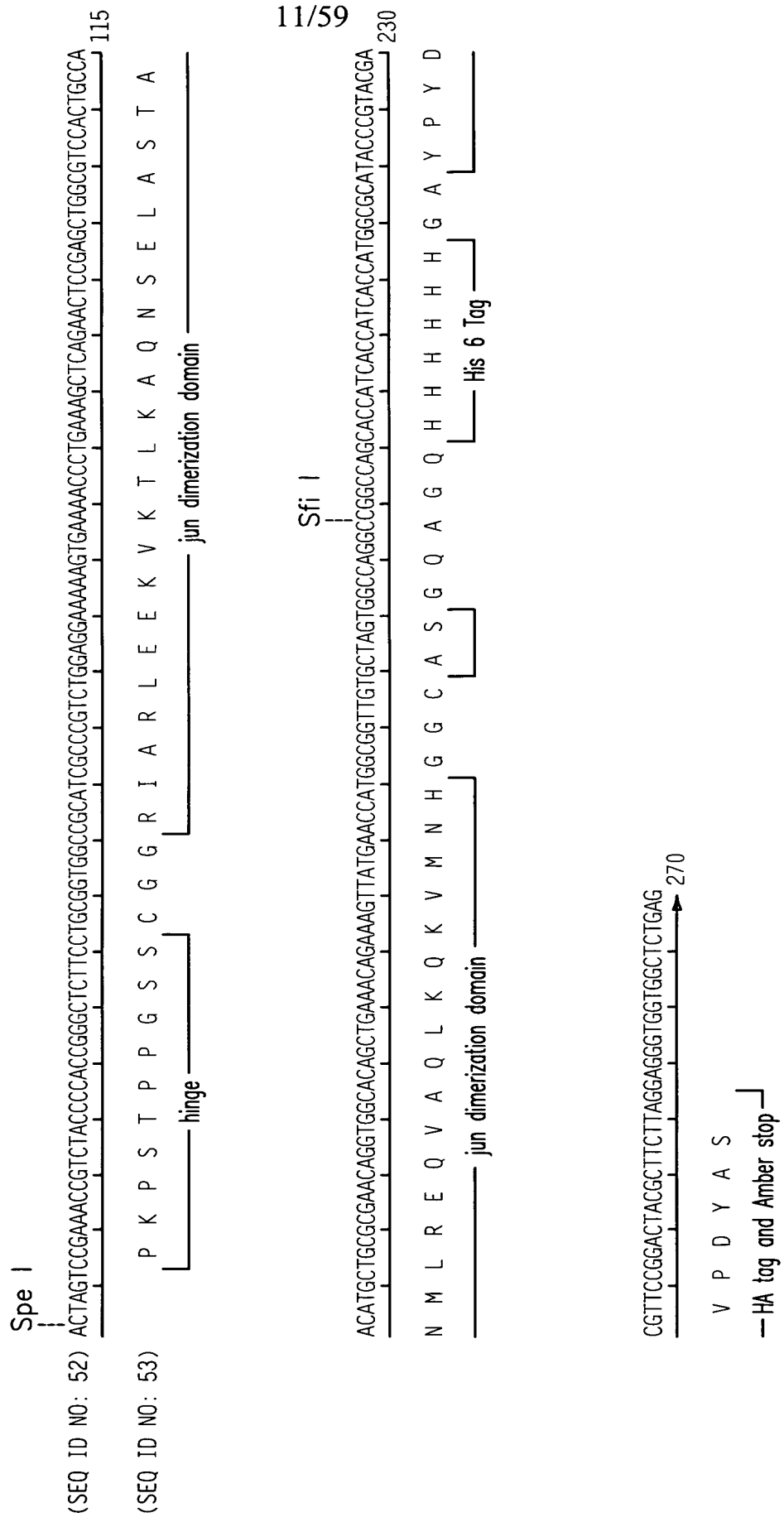


FIG. 8

TPO Positive Clones

nnk nnk 12/59

nnk nnk

1 2 IEGPTLRQWLAARA 3 4

	Sample	nnk	nnk	nnk	nnk	Amino Acids		Amino Acids	
		1	2	3	4	1	2	3	4
HC CDR3	X1c	tgg	ctg	cct	gtc	Trp	Leu	Pro	Val
	X3a	gtg	gta	cct	gtt	Val	Val	Pro	Val
	X3b	ggg	gta	ccc	gat	Gly	Pro	Pro	Asp
	X4b	ttg	cca	cct	gtt	Leu	Pro	Pro	Val
	X4c	tca	ctg	ccc	atc	Ser	Leu	Pro	Ile
	X5a	aca	atg	ccc	gtt	Thr	Met	Pro	Val
	X5c	acg	aca	cct	gtc	Trp	Leu	Pro	Val
	X7c	cag	aca	cct	cac	Gln	Thr	Pro	Asp
HC CDR2	24	ctt	tat	tct	aat	Leu	Tyr	Ser	Asn
	39	act	tac	ttg	cat	Thr	Tyr	Leu	His
	3	agg	atg	ctc	gag	Arg	Met	Leu	Glu
	7	aag	gaa	tct	aag	Lys	Glu	Ser	Lys
	8	gcg	cat	gtg	cag	Ala	His	Val	Gln
	10	cag	gag	att	agt	Gln	Glu	Ile	Ser
	11	cgg	aat	aat	ccg	Arg	Asn	Asn	Pro
	19	cag	cta	aat	tct	Gln	Leu	Asn	Ser
	25	agt	att	ttt	gtc	Ser	Ile	Phe	Val
	28	ggg	ccc	act	agt	Gly	Pro	Thr	Ser
LC CDR1	10	aag	ggt	gtt	agt	Lys	Gly	Val	Ser
	11	cat	ggg	gtg	gct	His	Gly	Val	Ala
	12a	cgt	acg	atg	gct	Arg	Thr	Met	Ala
	12b	cgt	ggt	gtt	aat	Arg	Gly	Val	Asn
	14	cgt	tcg	ctt	gcg	Arg	Ser	Leu	Ala
	16	cgg	ggt	gtt	gcg	Arg	Gly	Val	Ala
	18	agg	acg	gtg	tct	Arg	Thr	Val	Ser
	47	aag	ggg	gtg	gcg	Lys	Gly	Val	Ala
LC CDR2	1	aat	ccg	agg	ggt	Asn	Pro	Arg	Gly
	2	tcg	cct	cgg	agt	Ser	Pro	Arg	Ser
	3	tcg	cct	cgt	acg	Ser	Pro	Arg	Thr
	4	tcg	cct	tgg	cgt	Ser	Pro	Trp	Arg
	5	act	ccg	aat	tgg	Thr	Pro	Asn	Trp
	6	aat	cct	gcg	agg	Asn	Pro	Ala	Arg
	7	aat	ccg	tcg	ggg	Asn	Pro	Ser	Gly
	9	aat	cct	tat	tag	Asn	Pro	Tyr	Stop
	10	aat	ccg	cgg	tct	Asn	Pro	Arg	Ser
	11	aat	ccg	gat	gtg	Asn	Pro	Asp	Val
	12	tcg	ccg	tcg	cgg	Ser	Pro	Ser	Arg
	13	aat	cct	ctg	ttt	Asn	Pro	Leu	Phe
	14	aat	ctt	ggg	tat	Asn	Pro	Gly	Tyr
	15	aat	cct	att	agt	Asn	Pro	Ile	Ser
	16	aat	cct	cag	cgg	Asn	Pro	Gln	Arg
	18	aat	ccg	cgg	acg	Asn	Pro	Arg	Thr
	19	aat	ccg	cgt	ggg	Asn	Pro	Arg	Gly
	20	cat	ttg	aga	ctg	His	Leu	Arg	Leu
	21	aag	tag	att	tat	Lys	Stop	Ile	Tyr
	23	aat	cct	ggt	aag	Asn	Pro	Gly	Lys
	24	aat	cct	cgt	ggg	Asn	Pro	Arg	Gly
	26	aat	cct	aat	gtg	Asn	Pro	Asn	Val
	27	tct	ccg	cgg	gtt	Ser	Pro	Arg	Val
	29	acg	cct	cgg	ggt	Thr	Pro	Arg	Gly
	30	?ct	tag	tgg	tgg	Pro	Stop	Trp	Trp

FIG. 9

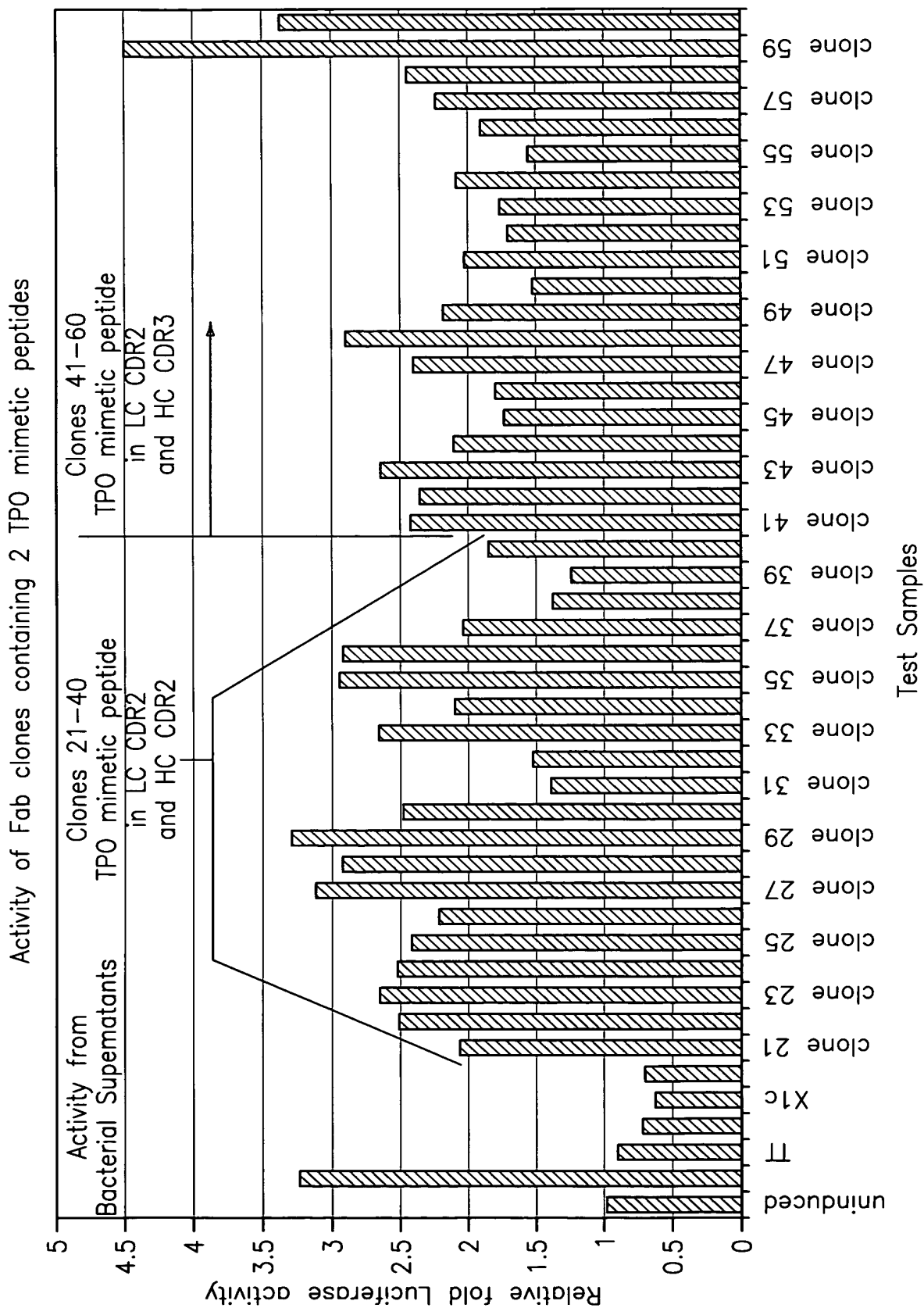


FIG. 10

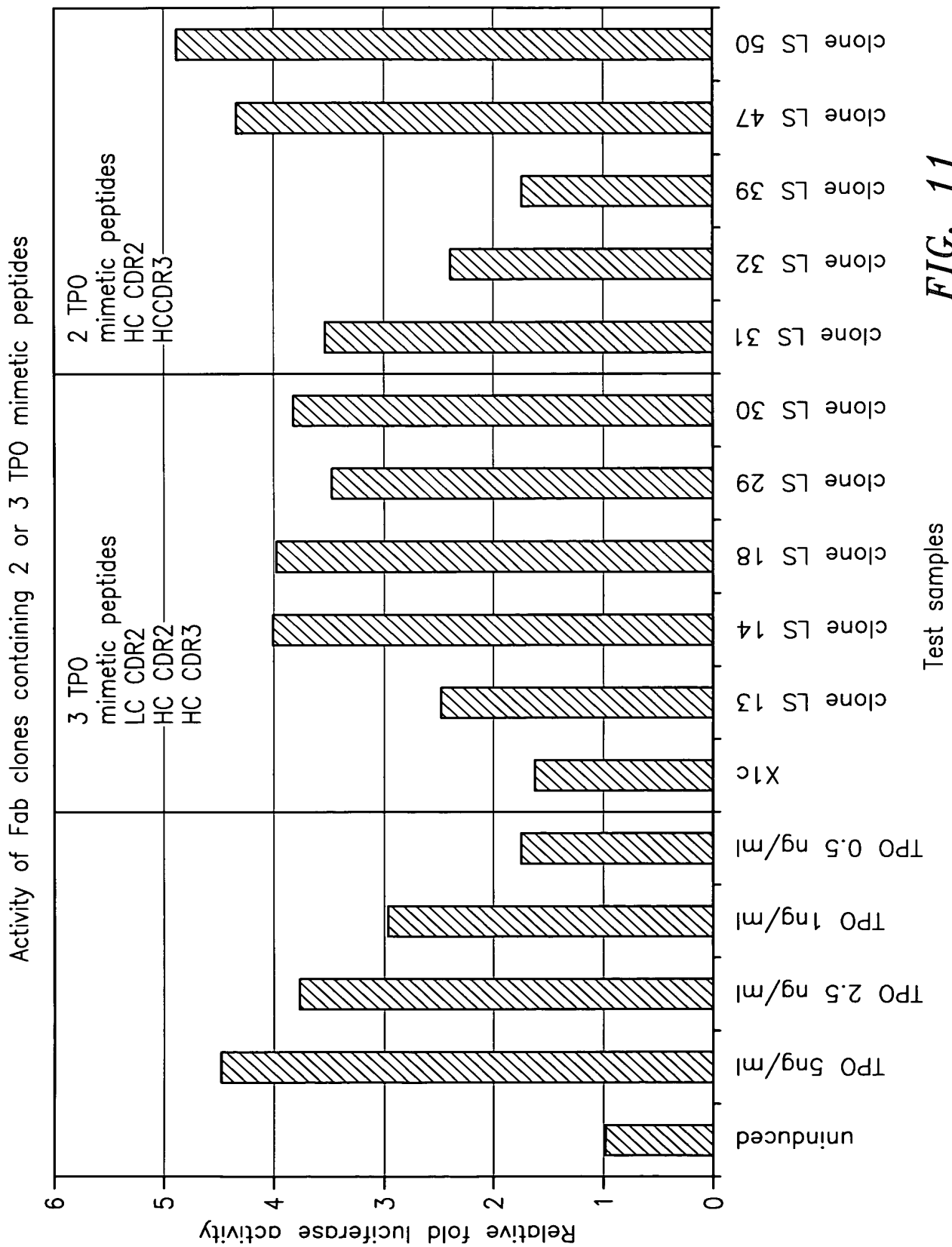


FIG. 11

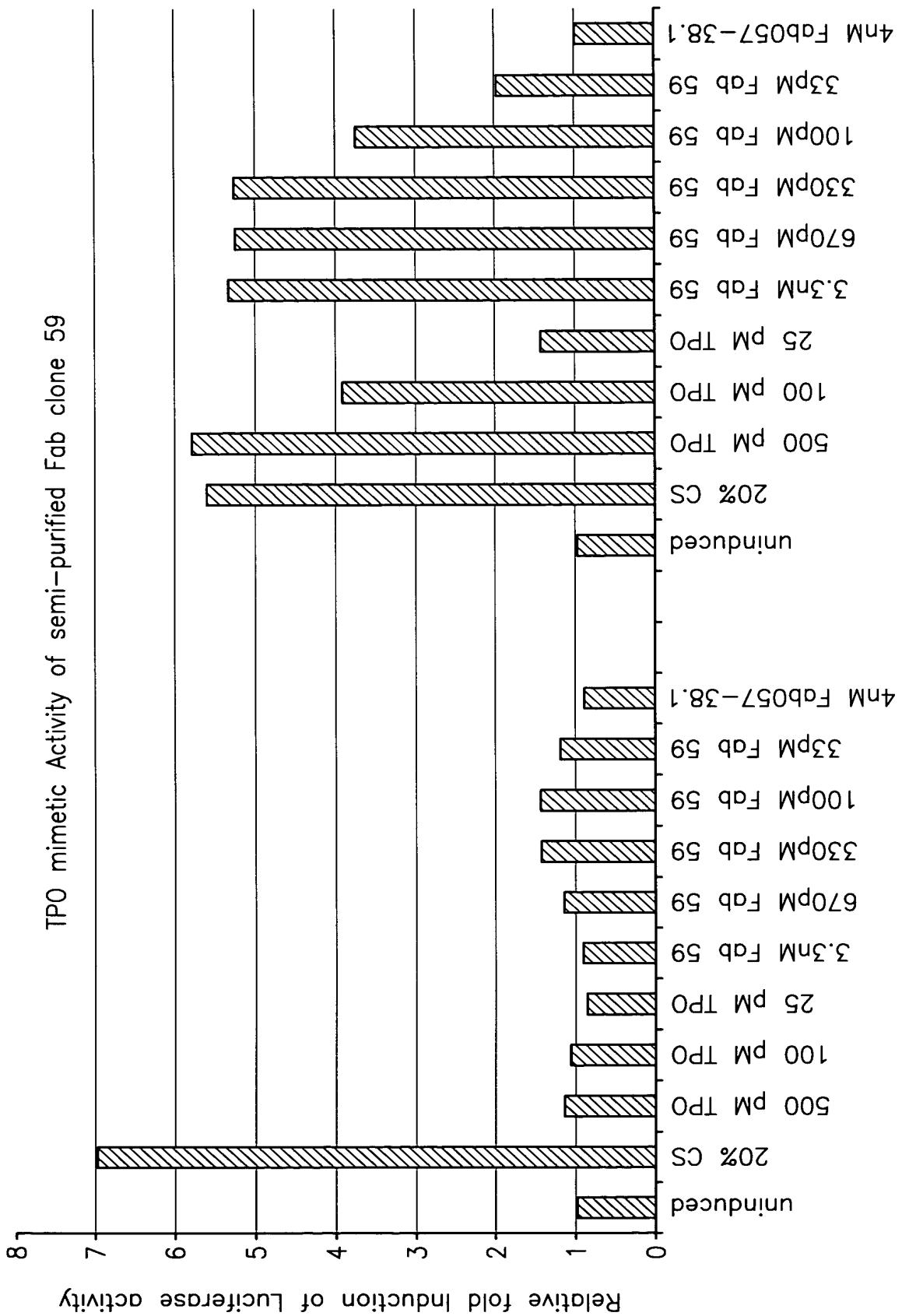


FIG. 12

(SEQ ID NO: 67)

5G1.1-TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:

MKWSWVILFLLSVTAGVHSQVQLVQSGAEVKKPGASVKVSCKASGYIFSNIWIQW
 VRQAPGQGLEWMGEILPGSGSTEYTENFKDRVTMTRDTSTSTVYMELSSLRSED
 TAVYYCARLPPIEGPTLRQWLAARAPVWGQGLTVTVSSASTKGPSVFPLAPCSR
 STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSNFGTQTYTCNVDPKPSNTKVDKTKVERKCCVECPPCPAPPVAGPSVFLFPPKP
 KDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQ
 EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRL
 TVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK.

(SEQ ID NO: 68)

5G1.1-TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:

ATGAAGTGGAGCTGGGTATTCTCTTCCTCCTGTCAGTAACTGCCGGCGTCCA
 CTCCCAAGTCCAAGTGGTGAATCCGGCGCCGAGGTCAAGAAGCCAGGGGCC
 TCAGTCAAAGTGTCTGTAAAGCTAGCGGCTATATTTTTTCTAATTATTGGAT
 TCAATGGGTGCGTCAGGCCCGGGCAGGGCCTGGAATGGATGGGTGAGATC
 TTACCGGGCTCTGGTAGCACCGAATATACCGAAAATTTTAAAGACCGTGTTA
 CTATGACGCGTGACACTTCGACTAGTACAGTATACATGGAGCTCTCCAGCCTG
 CGATCGGAGGACACGGCCGTCTATTATTGCGCGCGTTTGCCAATTGAAGGG
 CCGACGCTGCGGCAATGGCTGGCGGCGCGCGCGCCTGTTTGGGGTCAAG
 GAACCTGGTCACTGTCTCGAGCGCCTCCACCAAGGGCCCATCCGTCTTCCCC
 CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGTGCG
 TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGCTGGAAGTCAAGCGC
 CCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
 ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGAC
 CTACACCTGCAACGTAGATCACAAGCCAGCAACACCAAGGTGGACAAGAC
 AGTTGAGCGCAAATGTTGTGTCGAGTGCCACCGTGCCAGCACCACCTGTG
 GCAGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCATCATGAT
 CTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC
 CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCA
 AGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCG
 TCCTCACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAA
 GGTCTCCAACAAAGGCCTCCCGTCTCCATCGAGAAAACCATCTCCAAGCC
 AAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAG
 GAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTACC
 CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
 ACAAGACCACGCCTCCCGTCTGGACTCCGACGGCTCCTTCTTCTCTACAGC
 AGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCT
 CCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCT
 GTCTCTGGGTAAATGA

(SEQ ID NO: 69)

5G1.1 Light Chain Amino Acid Sequence

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVITITCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

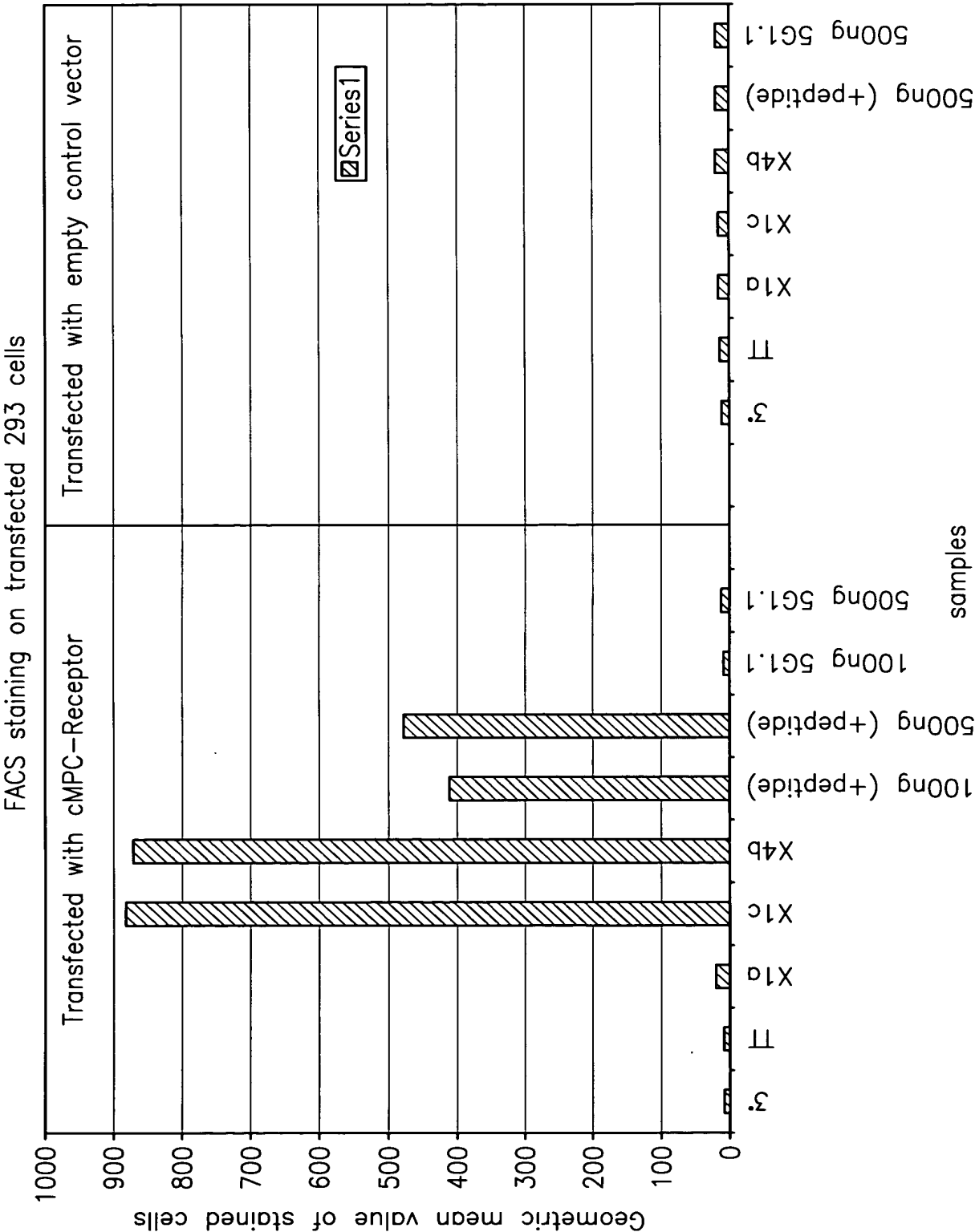
(SEQ ID NO: 70)

5G1.1 Light Chain Nucleic Acid Sequence

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGTCCTCCCTGTCCGCCT
CTGTGGGCGATAGGGTCACCATCACCTGCGGCGCCAGCGAAAACATCTATGG
CGCGCTGAACTGGTATCAACAGAAACCCGGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCGACGAACCTGGCAGATGGAGTCCCTTCTCGCTTCTCTGGATCCGG
CTCCGGAACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAATAAAACGAACGTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTAG

Note: Italics denotes leader sequence

FIG. 13B



samples
FIG. 14

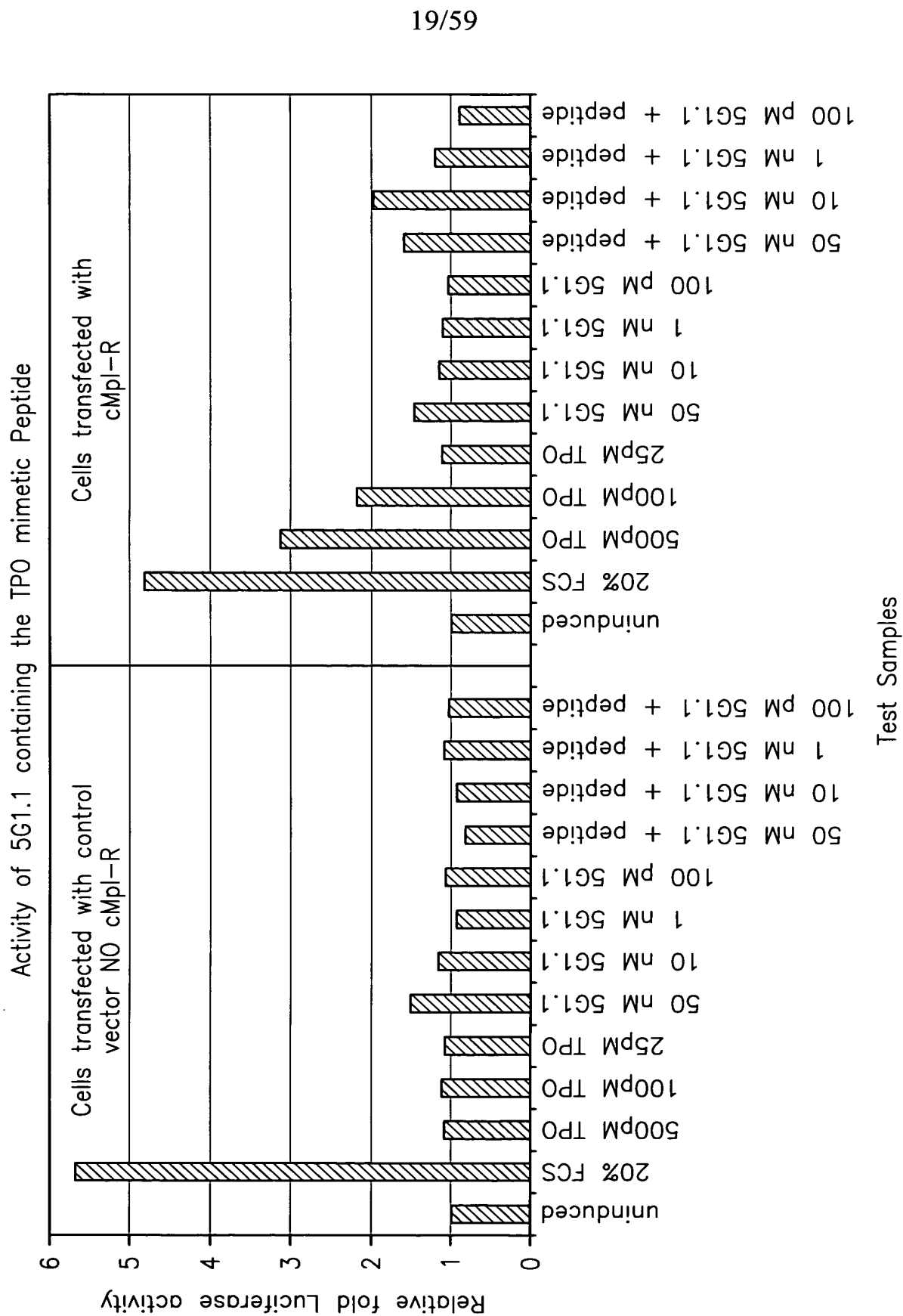


FIG. 15

20/59

CDR1

CDR1

Grafted CDR2

Grafted CDR2

CDR3

TAT	TAT	TAC	TGC	CAA	CAG	TAT	AAT	AGT	TAC	CCT	CCC	ACT	TTC	GGC	CCT	GGG	ACC	AAA	AAA	GTG	GAT	ATC	AAA
86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107		

FIG. 16

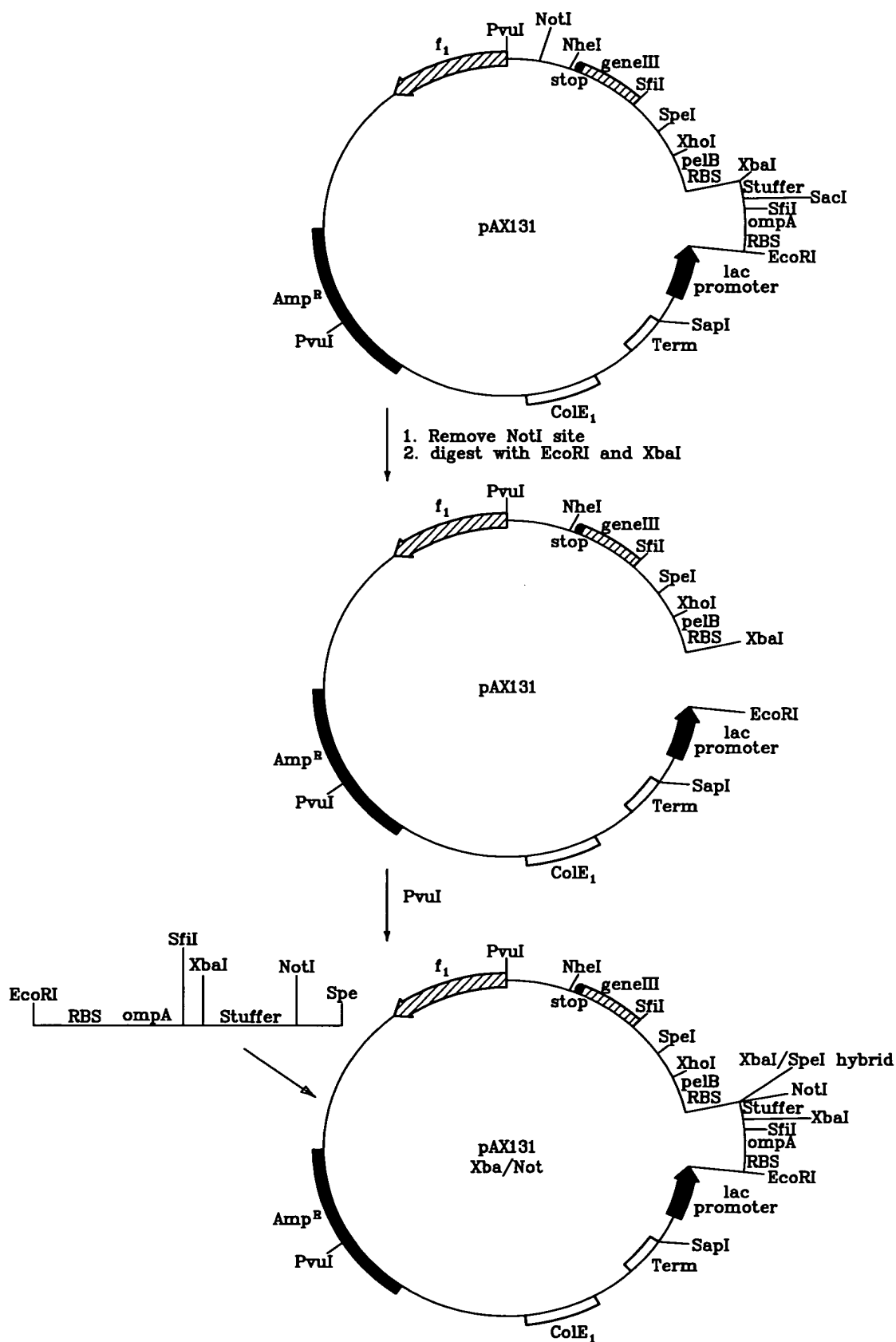


FIG. 17

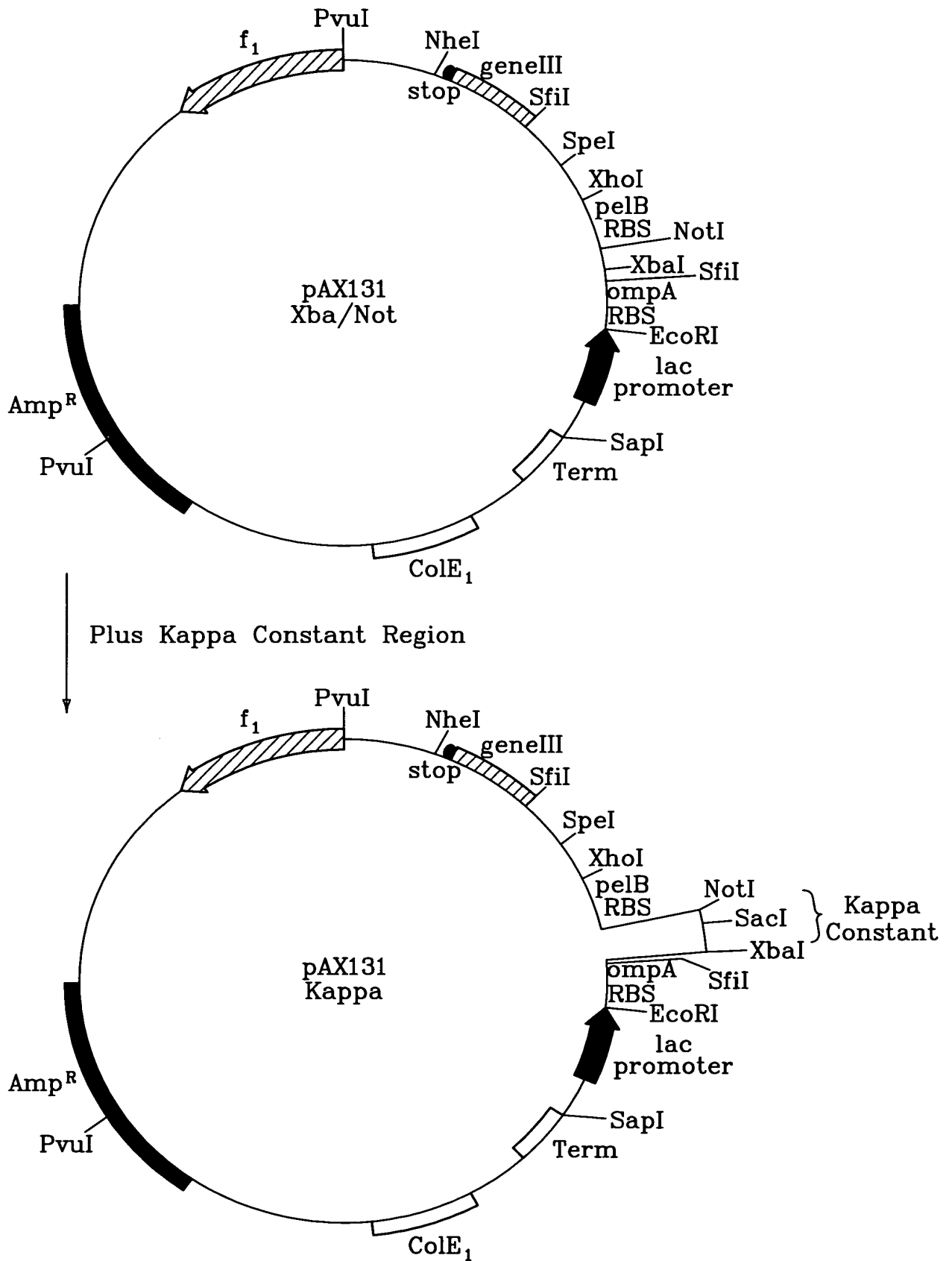
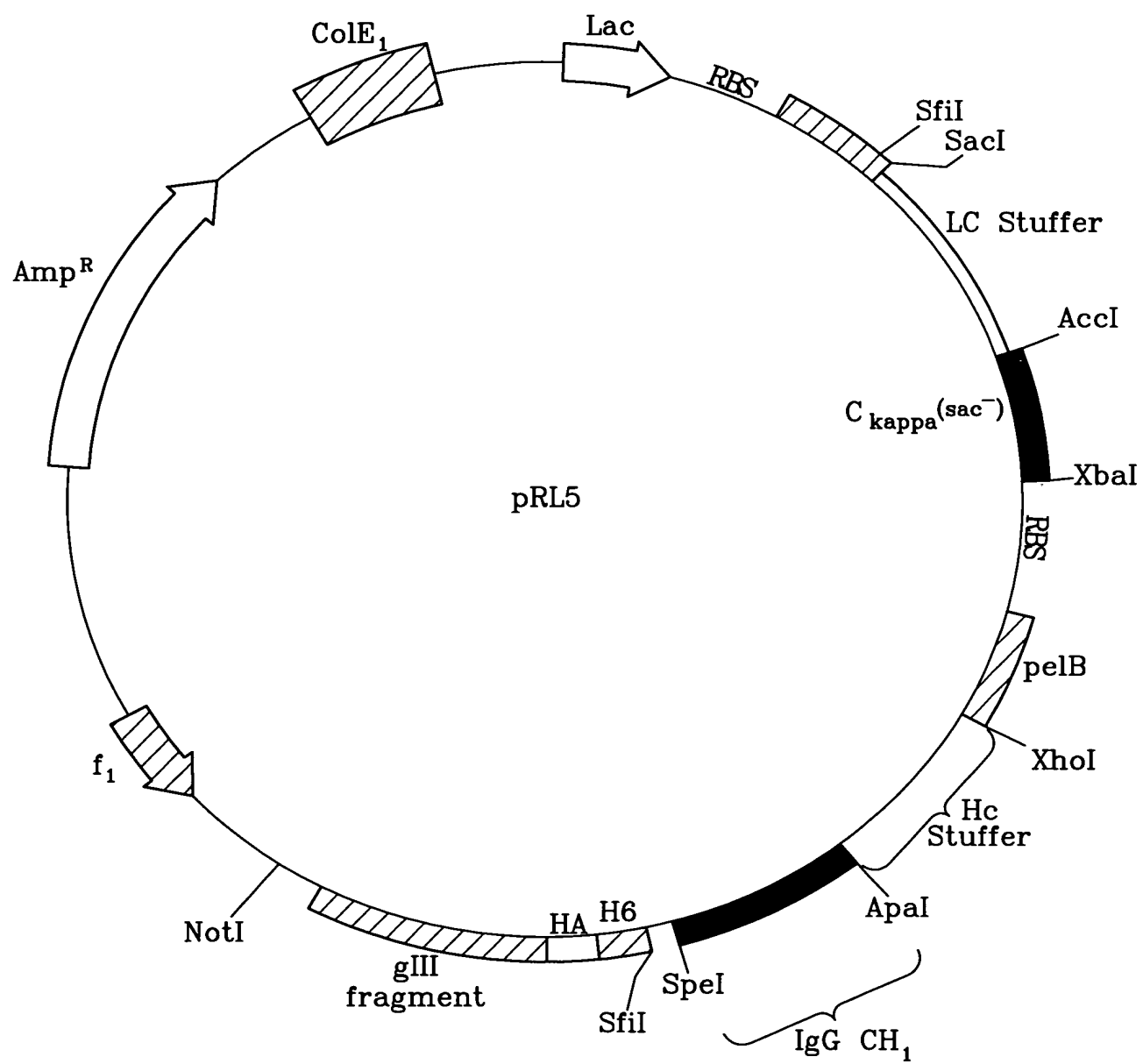


FIG. 18

*FIG. 19*

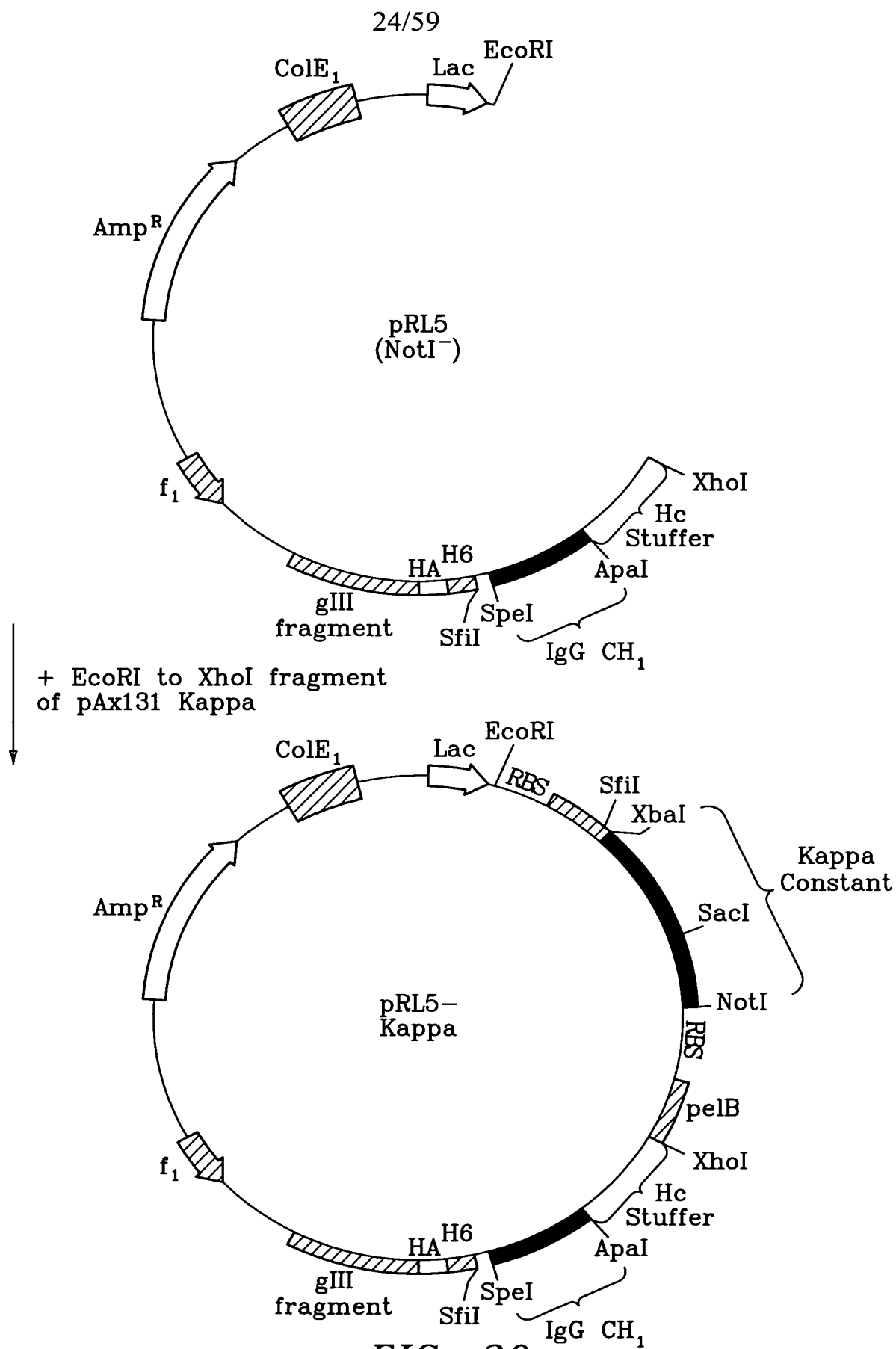
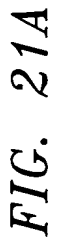


FIG. 20



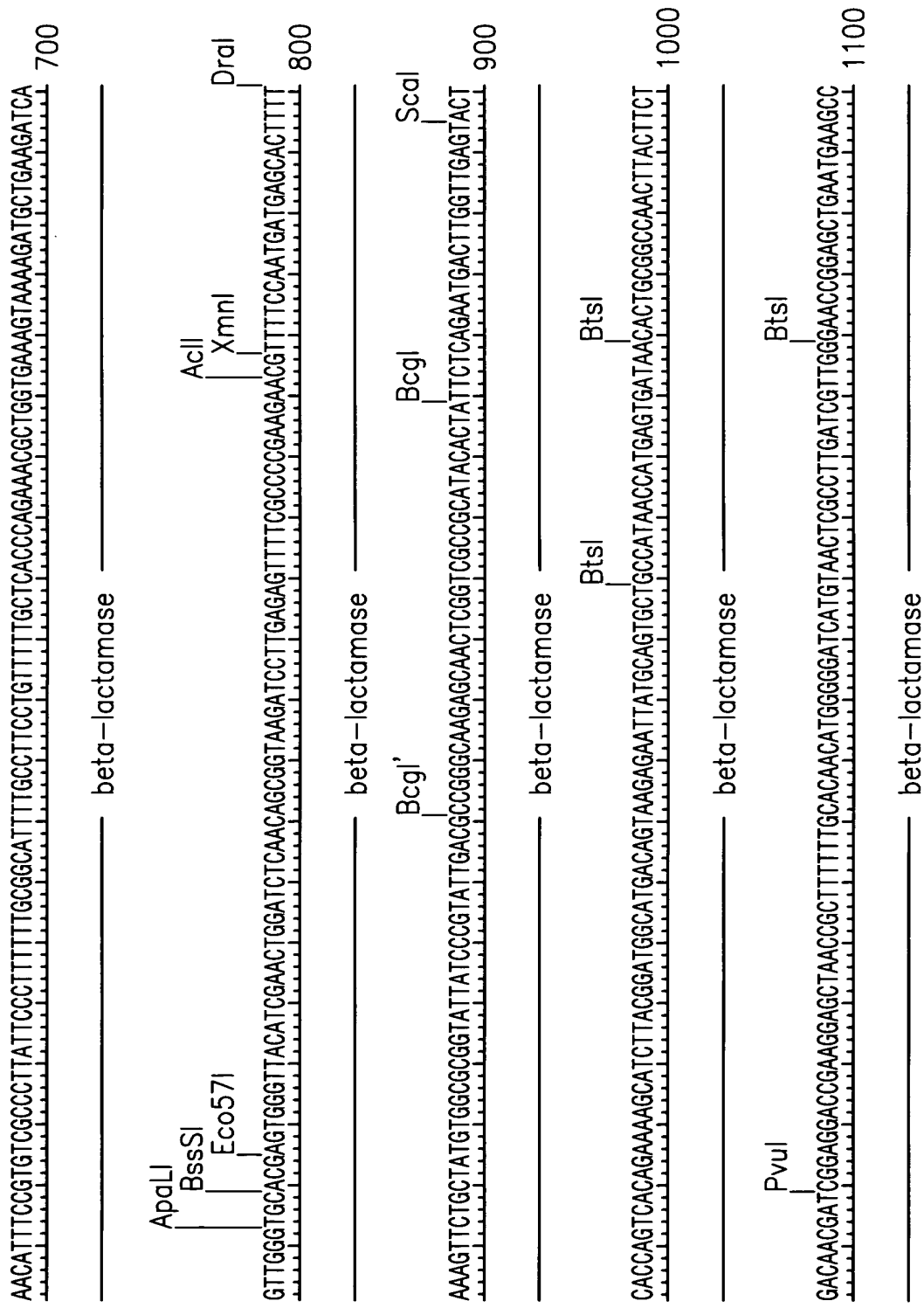


FIG. 21B

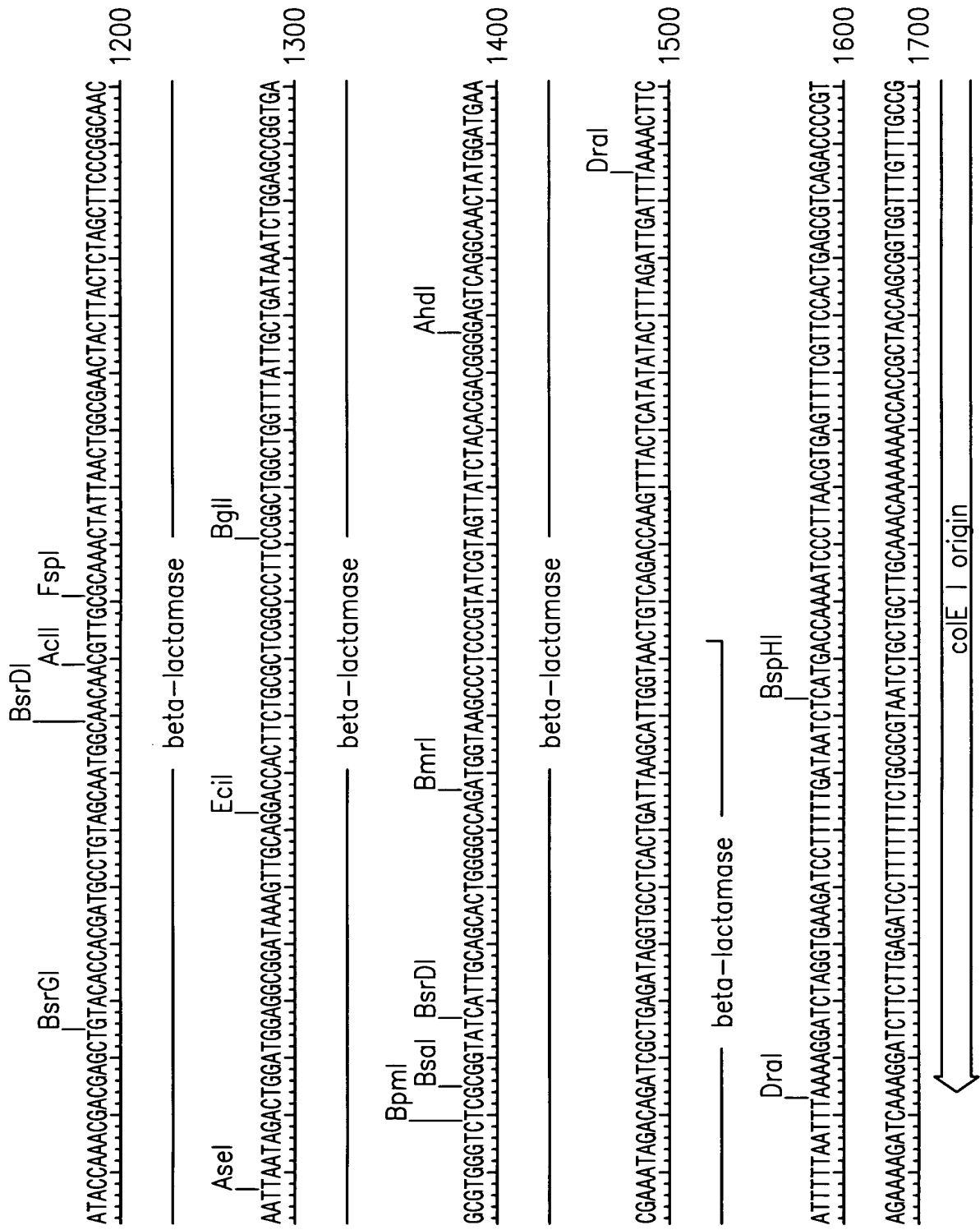


FIG. 21C

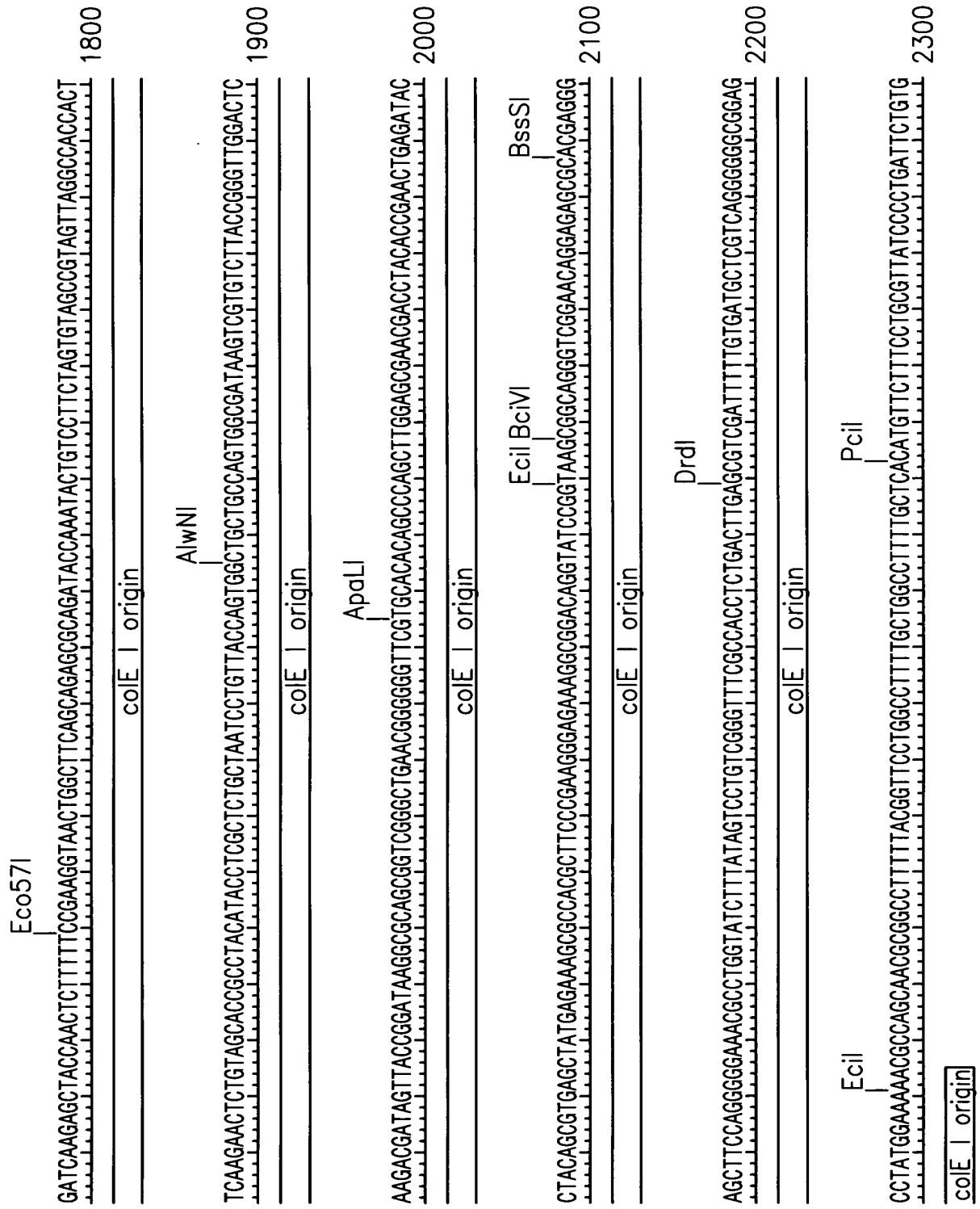


FIG. 21D

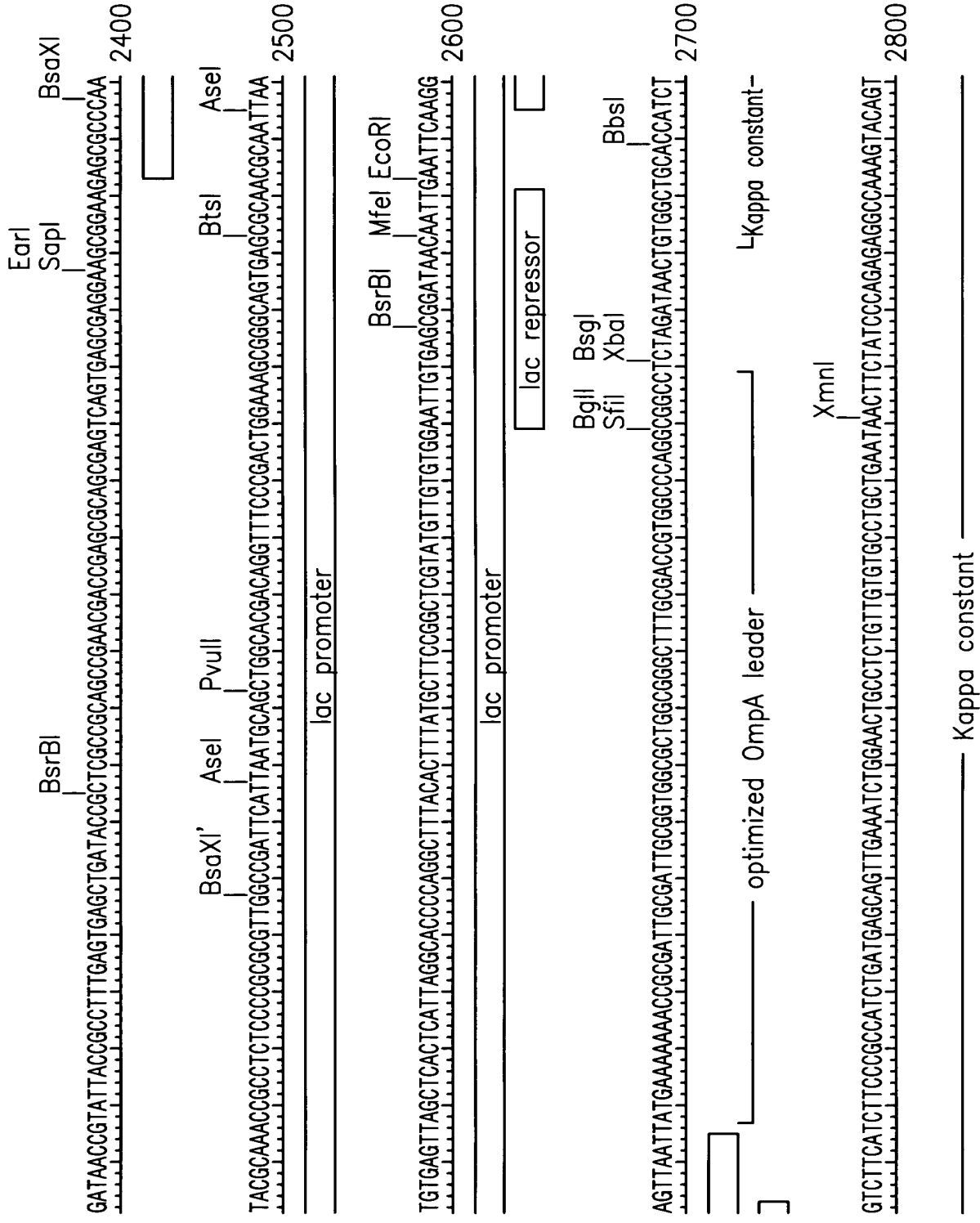


FIG. 21E

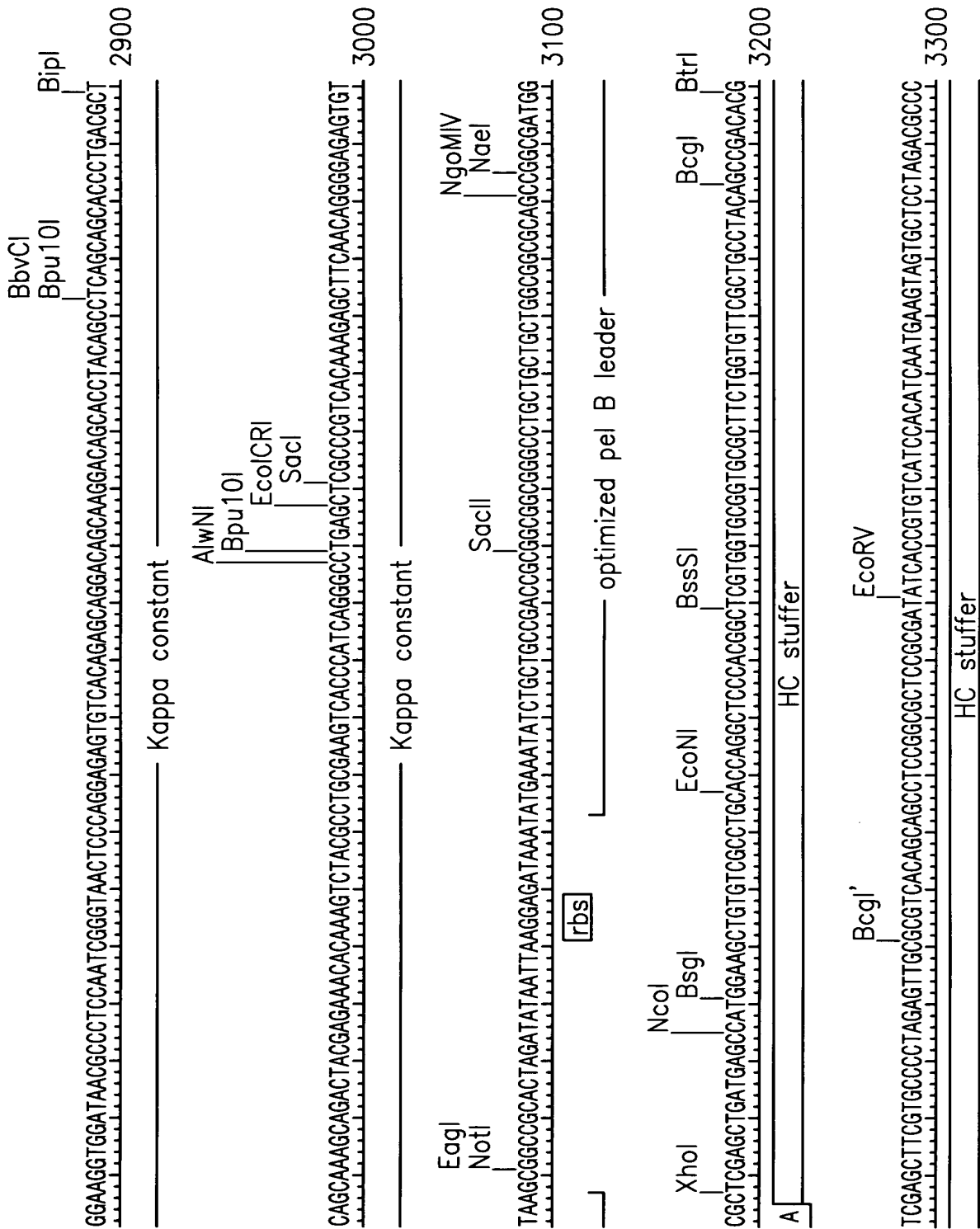


FIG. 21F

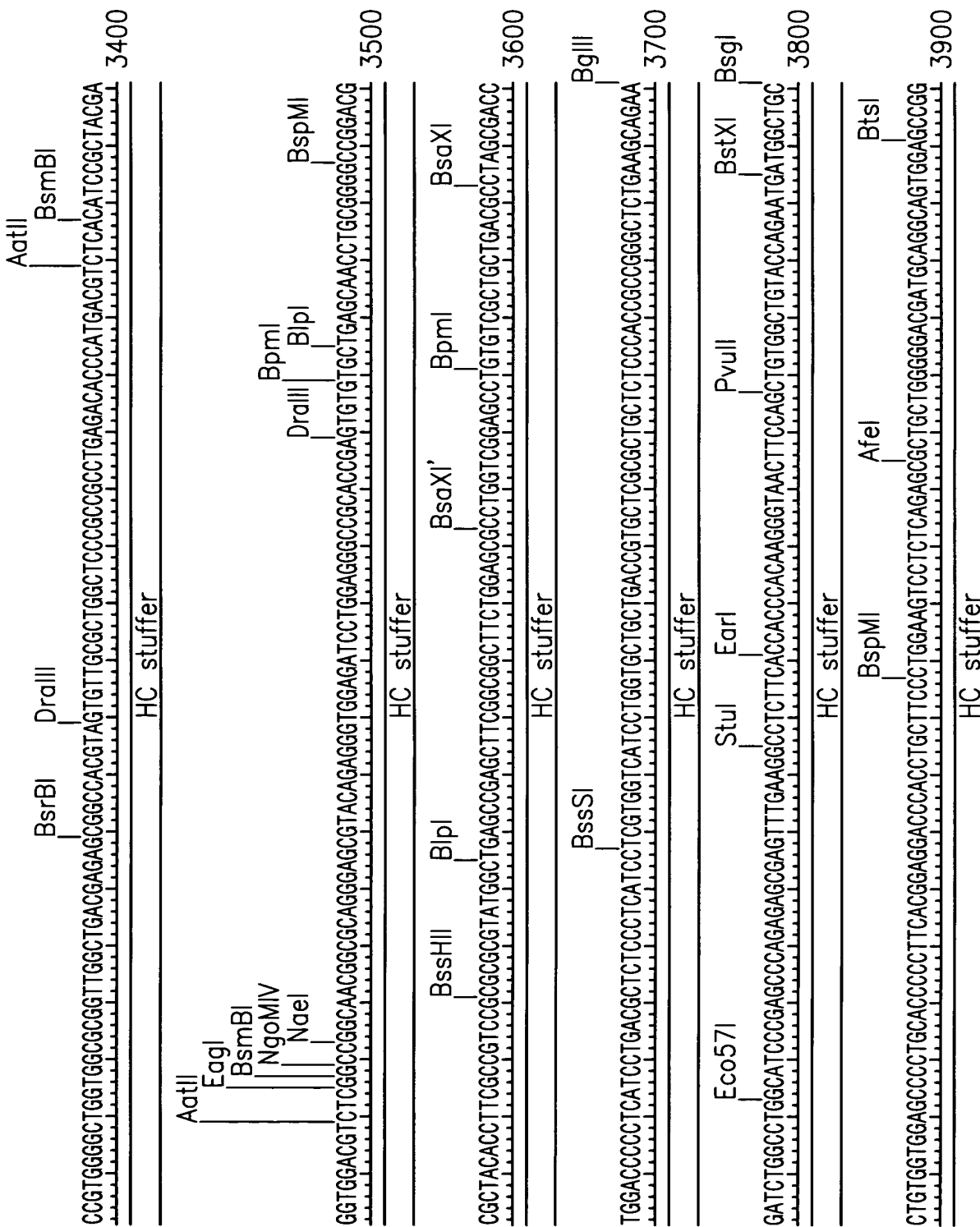


FIG. 21G

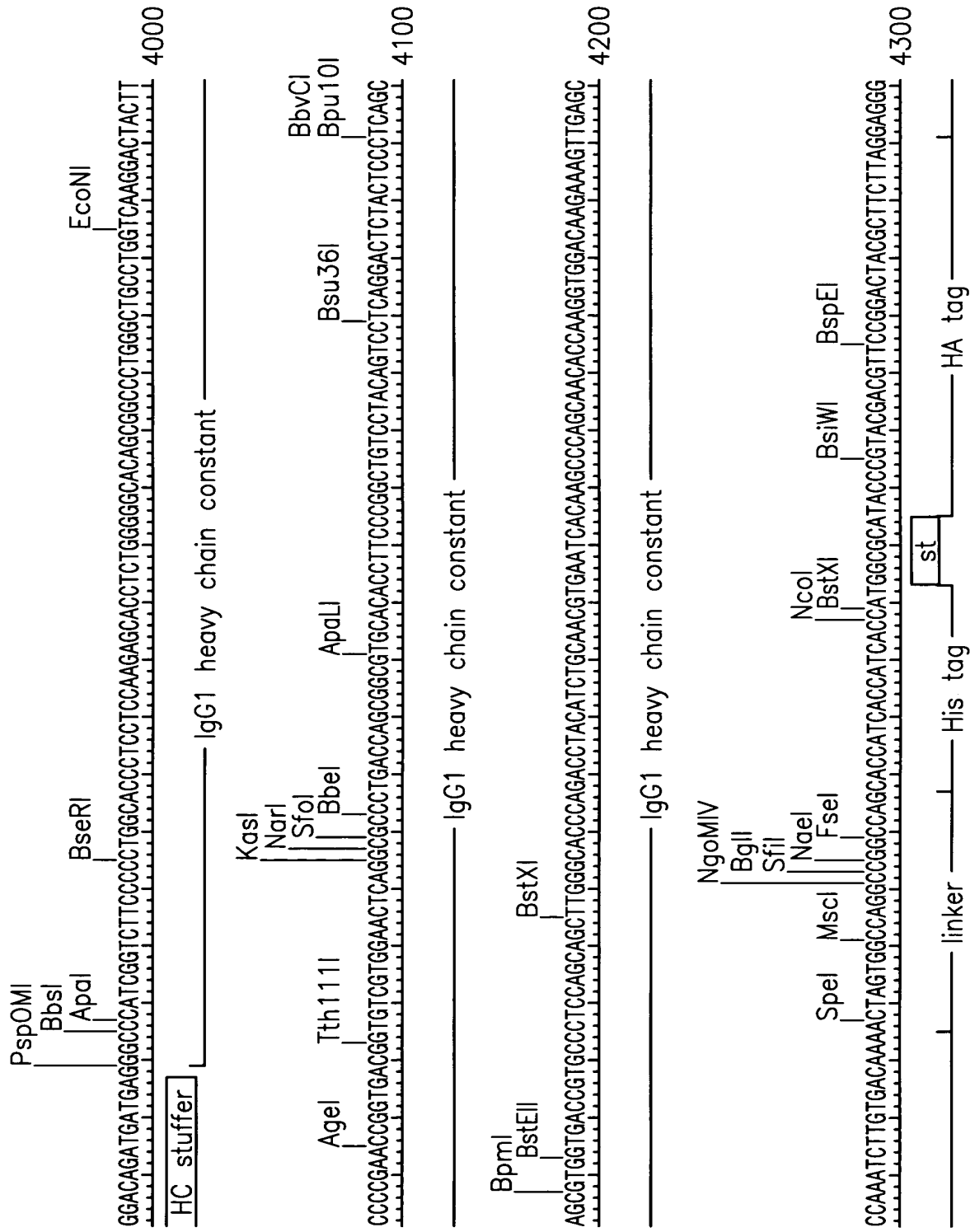


FIG. 21H

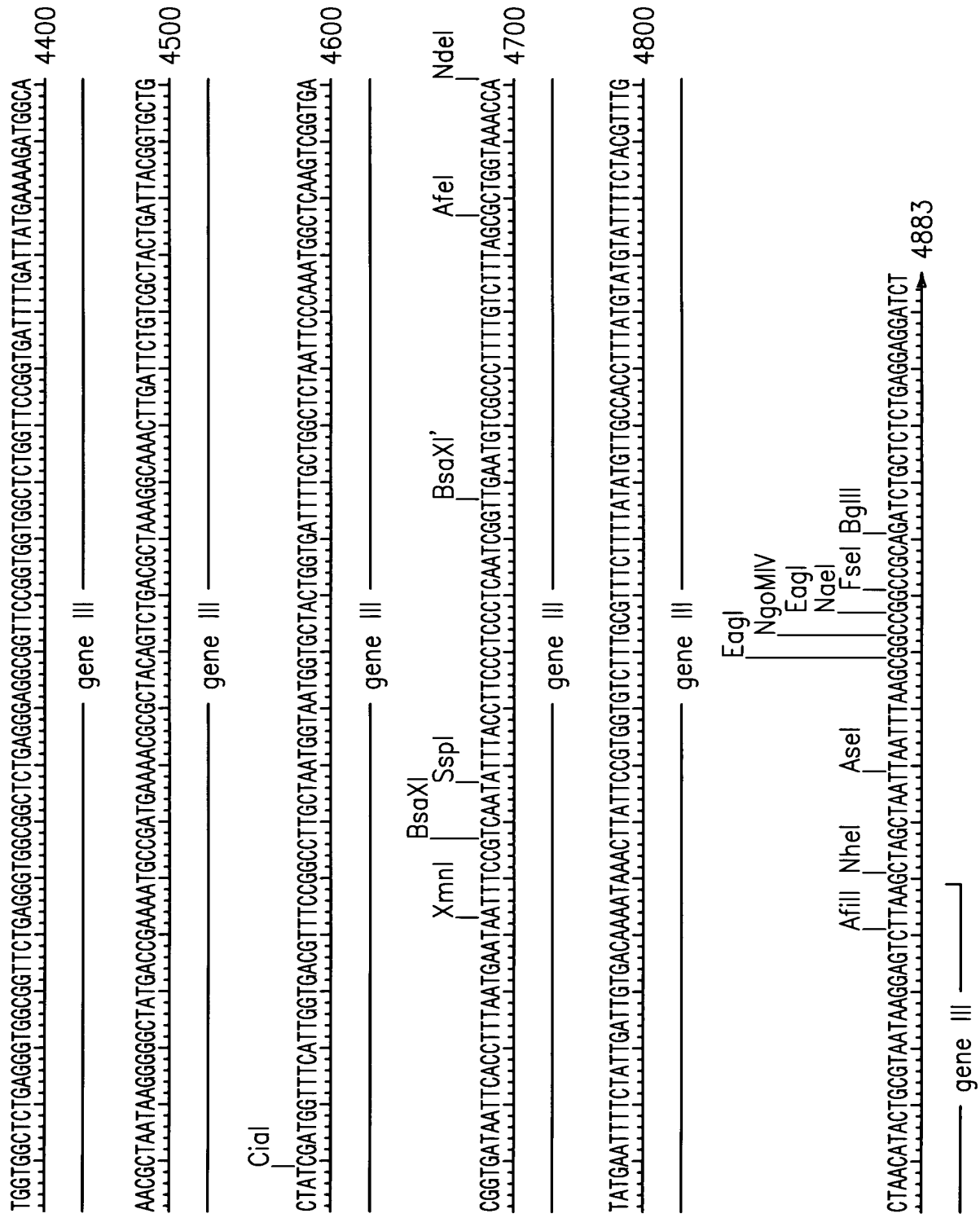


FIG. 211

VH: L22582 (human germline family member VH1-69)

GCAGGATTTAGGGCTTGGTCTCTCAGCATCCCACACTTGTACAGCTGATGTGGCATCTG
 TGTTTTCTTTCTCATCGTAGATCAGGCTTTGAGCTGTGAAATACCCTGCCTCATGCATATGCA
 AATAACCTGAGGTCTTCTGAGATAAATATAGATATATTGGTGCCCTGAGAGCATCACATAA
 CAACCACATTCCTCCTCTAAAGAAGCCCCTGGGAGCACAGCTCATCACCATGGACTGGACC
 TGGAGGTTCTCTTTGTGGTGGCAGCGCTACAGGTAAGGGGCTTCCTAGTCCTAAGGCTGAG
 GAAGGGATCCTGGTTTAGTTAAAGAGGATTTTATTCACCCCTGTGTCTCTCCACAGGTGTC
 CAGTCCCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGA
 AGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACA
 GGCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAAC
 TACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCT
 ACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGACAC
 AGTGTGAAAACCCACATCCTGAGAGTGTGAGAAACCCTGAGGGAGAAGGCAGCTGTGCCG
 GGCTGAGGAGATGACAGGGTTTATTAGGTTTAAGGCTGTTTACAAAATGGGTATATATTTG
 AGAAAAAAGAACAGTAGAAACAAGTACATACTCCTCTAATTTTAAGATAATTATTCATT
 CAAGAGTCGTAATAT (SEQ ID NO: 153)

JH---JH6

H3

 CDR3

100

110

JH6 YYYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 154)

FIG. 22

Vk: X12686 (human germline family member VKIII-A27)

CAGCTGCTTTGCATGTCCCTCCCAGCCGCCCTGCAGTCCAGAGCCCATATCAATGCCTGG
 GTCAGAGCTCTGGAGAAGAGCTGCTCAGTTAGGACCCAGAGGGAACCATGGAAACCCCAG
 CGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGGTGAGGGGAACATGGGATGGTTTTGC
 ATGTCAGTGAAAACCTCTCAAGTCCTGTTACCTGGCAACTCTGCTCAGTCAATACAATAAT
 TAAAGCTCAATATAAAGCAATAATTCTGGCTCTTCTGGGAAGACAATGGGTTTGATTTAGAT
 TACATGGGTGACTTTTCTGTTTTATTTCCAATCTCAGATACCACCGGAGAAATTGTGTTGAC
 GCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
 GTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGCAGT
 GGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGT
 ATTACTGTCAGCAGTATGGTAGCTCACCTCCCACAGTGATTTCAGCTTGAAACAAAAACCTCT
 GCAAGACCTTCATTGTTTACTAGATTATACCAGCTG (SEQ ID NO: 155)

JK

L3

-

CDR3

--

100

|

Jk1 WTFGQGTKVEIK (SEQ ID NO: 156)

FIG. 23

pAXB116 Fab' gVh

(SEQ ID NO: 157)																	
pelB leader																	
ATG	AAA	TAC	CTA	TTG	CCT	ACG	GCA	GCC	GCT	GGA	TTG	TTA	TTA	CTC	GCT	GCC	CAA
M	K	Y	L	L	P	T	A	A	A	G	L	L	L	L	A	A	Q
(SEQ ID NO: 158)																	
pelB leader																	
CCA	GCC	ATG	GCG	CAG	GTG	CAG	CTG	GTG	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG
P	A	M	A	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P
(SEQ ID NO: 159)																	
(SEQ ID NO: 169)																	
Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P				
L22582																	
CDR1-H1																	
GGC	AGC	AGC	GTG	AAA	GTG	AGC	TGC	AAA	GCG	AGC	GGC	GCG	ACC	TTT	AGC	AGC	TAT
G	S	S	V	K	V	S	C	K	A	S	G	G	T	F	S	S	Y
G	S	S	V	K	V	S	C	K	A	S	G	G	T	F	N	N	Y
G	S	S	V	K	V	S	C	K	A	S	G	G	T	F	S	S	Y
L22582																	
pAXB116 Fab'-gVh																	
TT-Vh (CDR3-TPO)																	
L22582																	
CDR2-TPO																	
CTG	ATT	GAA	GCG	CCG	ACC	CTG	CGC	CAG	TGG	CTG	GCG	GCG	CGC	GCG	AAC	AGC	CGC
L	I	E	G	P	T	L	R	Q	W	L	A	A	R	A	N	S	R
I	I	F	P	F	R	N	T	A	K	Y	A	Q	H	F	Q	G	R
I	I	P	I	F	G	T	A	N	Y	A	Q	K	F	Q	G	R	L22582
pAXB116 Fab'-gVh																	
TT-Vh (CDR3-TPO)																	
L22582																	
GTG	ACC	ATT	ACC	GCG	GAT	GAA	AGC	ACC	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC
V	T	I	T	A	D	E	S	T	S	T	A	Y	M	E	L	S	S
V	T	I	T	A	D	E	S	T	G	T	A	Y	M	E	L	S	S
V	T	I	T	A	D	E	S	T	S	T	A	Y	M	E	L	S	S
L22582																	
pAXB116 Fab'-gVh																	
TT-Vh (CDR3-TPO)																	
L22582																	
CTG	CGC	AGC	GAA	GAT	ACC	GCG	GTG	TAT	TAT	TGC	GCG	CGC	CTG	CCG	ATT	GAA	GCG
L	R	S	E	D	T	A	V	Y	Y	C	A	R	L	P	I	E	G
L	R	S	E	D	T	A	I	Y	Y	C	A	R	L	P	I	E	G
L	R	S	E	D	T	A	V	Y	Y	C	A	R					
L22582																	
CDR3-TPO																	
CCG	ACC	CTG	CGC	CAG	TGG	CTG	GCG	GCG	CGC	GCG	CCG	GTG	TGG	GGC	CAG	GGC	ACC
P	T	L	R	Q	W	L	A	A	R	A	P	V	W	G	Q	G	T
P	T	L	R	Q	W	L	A	A	R	A	P	V	W	G	Q	G	T
L22582																	
pAXB116 Fab'-gVh																	
TT-Vh (CDR3-TPO)																	
L22582																	
ACC	GTG	ACC	GTG	AGC	AGC												
T	V	T	V	S	S												
T	V	T	V	S	A												
L22582																	
pAXB116 Fab'-gVh																	
TT-Vh (CDR3-TPO)																	

Sequence of the pAXB116 Fab' Heavy chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. TPO peptides in heavy chain CDR2 and CDR3 of pAXB116 Fab' are indicated by double underlines and wavelines respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVh denotes heavy chain variable region of human germline derived pAXB116 Fab'.

FIG. 24

pAXB116 Fab' gVk

(SEQ ID NO: 160)		<u>pelB leader</u>																	
ATG	AAA	TAC	CTA	TTG	CCT	ACG	GCA	GCC	GCT	GGA	TTG	TTA	TTA	CTC	GCT	GCC	CAA		
M	K	Y	L	L	P	T	A	A	A	G	L	L	L	L	A	A	Q		
(SEQ ID NO: 161)		<u>pelB leader</u>																	
CCA	GCC	ATG	GCG	GAA	ATT	GTG	CTG	ACC	CAG	AGC	CCG	GGC	ACC	CTG	AGC	CTG	AGC		
P	A	M	A	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	pAXB116 Fab'-gVk	
(SEQ ID NO: 162)																		TTVk	
(SEQ ID NO: 170)																		X12686	
		<u>CDR1-L1</u>																	
CCG	GGC	GAA	CGC	GCG	ACC	CTG	AGC	TGC	<u>CGC</u>	<u>GCG</u>	<u>AGC</u>	<u>CAG</u>	<u>AGC</u>	<u>GTG</u>	<u>AGC</u>	<u>AGC</u>	<u>AGC</u>		
P	G	E	R	A	T	L	S	C	R	A	S	Q	S	V	S	S	S	pAXB116 Fab'-gVk	
P	G	E	R	A	T	L	S	C	R	A	S	H	S	V	S	R	A	TTVk	
P	G	E	R	A	T	L	S	C	R	A	S	Q	S	V	S	S	S	X12686	
		<u>CDR2-L2</u>																	
TAT	CTG	GCG	TGG	TAT	CAG	CAG	AAA	CCG	GGC	CAG	GCG	CCG	CGC	CTG	CTG	ATT	TAT		
Y	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	pAXB116 Fab'-gVk	
Y	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	TTVk	
Y	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	X12686	
		<u>CDR3-L3</u>																	
GGC	GCG	AGC	AGC	CGC	GCG	ACC	GGC	ATT	CCG	GAT	CGC	TTT	AGC	GGC	AGC	GGC	AGC		
G	A	S	S	R	A	T	G	I	P	D	R	F	S	G	S	G	S	pAXB116 Fab'-gVk	
G	T	S	S	R	A	T	G	I	P	D	R	F	S	G	S	G	S	TTVk	
G	A	S	S	R	A	T	G	I	P	D	R	F	S	G	S	G	S	X12686	
GGC	ACC	GAT	TTT	ACC	CTG	ACC	ATT	AGC	CGC	CTG	GAA	CCG	GAA	GAT	TTT	GCG	GTG		
G	T	D	F	T	L	T	I	S	R	L	E	P	E	D	F	A	V	pAXB116 Fab'-gVk	
G	T	D	F	T	L	T	I	S	R	L	E	P	E	D	F	A	V	TTVk	
G	T	D	F	T	L	T	I	S	R	L	E	P	E	D	F	A	V	X12686	
		<u>CDR3-L3</u>																	
TAT	TAT	TGC	<u>CAG</u>	<u>CAG</u>	<u>TAT</u>	<u>GGC</u>	<u>AGC</u>	<u>AGC</u>	<u>CCG</u>	<u>TGG</u>	<u>ACC</u>	TTT	GGC	CAG	GGC	ACC	AAA		
Y	Y	C	Q	Q	Y	G	S	S	P	W	T	F	G	Q	G	T	K	pAXB116 Fab'-gVk	
Y	Y	C	Q	Q	Y	G	S	S	P	W		F	G	Q	G	T	K	TTVk	
Y	Y	C	Q	Q	Y	G	S	S	P				G	Q	G	T	K	X12686	
		<u>CDR3-L3</u>																	
GTG	GAA	ATT	AAA																
V	E	I	K															pAXB116 Fab'-gVk	
V	E	L	K															TTVk	

Sequence of the pAXB116 Fab' Light chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVk denotes light chain variable region of human germline derived pAXB116 Fab'.

FIG. 25

Primers to generate pAXB116 heavy chain

UDEC1709 :5' primer 272 bp, containing NcoI site (SEQ ID NO: 163)

5'----CCAGCCATGGCGCAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTAAAAAACCGGGCAGCAGCGTGAAAGT
GAGCTGCAAAGCGAGCGGCGGCACCTTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGGCGCCGGGCCAGGGCCTG
GAATGGATGGGCGGCATTATTCGATTTTTGGCACCGCGAACTATGCGCAGAAATTTACAGGGCCGCGTGACCATTAC
CGCGGATGAAAGCACCAGCACCGCGTATATGGAAGTGCAGCAGCTGCG---3'

Overlapping with UDEC1710

UDEC1710 3' primer 271 bp

5'----GTTCCAGCTCACGGTCACCGTTCCGGAAAATAATCTTTCACCAGGCAGCCAGCGCCGCGGTGCCGCCG

Overlapping with UDEC1711

CTGGTGCTTTTGCTGCTCGGCGCCAGCGGAAACACGCTCGGGCCTTTGGTGCTCGCGCTGCTCACGGTCACGGTGGT
GCCCTGGCCCCACACGGCGCGCGCGCCGCCAGCCACTGGCGCAGGGTCGGGCCTTCAATCGGCAGGCGCGCGCAA
TAATACACCGCGGTATCTTCGCTGCGCAGGCTGCTCAGTTCCATATAC----3' (SEQ ID NO: 164)

Overlapping with UDEC1709

UDEC1711 3' primer (274 bp) containing XbaI site

5'----CGAGTCTAGATTACGGGCCGCCAGCAGTTCGGGCGCCGGGCACGGCGGGCAGGTATGGGTTTTATCGCAGCT
TTTCGGTTCCACTTTTTTATCCACTTTGGTGTTGCTCGGTTTATGGTTCACGTTGCAAATATAGGTCTGGGTGCCCAGG
CTGCTGCTCGGCACGGTCACCACGCTGCTCAGGCTATACAGGCCGCTGCTCTGCAGCACCGCCGGAAGGTATGCAC
GCCGCTGGTCAGCGCGCCGCTGTTCCAGCTCACGGTCACCGGTTTC----3' (SEQ ID NO: 165)

Overlapping with UDEC1710

FIG. 26

Primers to generate pAXB116 light chain

UDEC1712 5' primer 236 bp

5'---CCAGCCATGGCGGAAATTGTGCTGACCCAGAGCCCGGGCACCCTGAGCCTGAGCCCGGGCGAACGCGCGAC
CCTGAGCTGCCGCGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCG
CGCCTGCTGATTTATGGCGCGAGCAGCCGCGCAGCGGCATTCCGGATCGCTTTAGCGGCAGCGGCAGCGGCACCG
ATTTTACCCTGAC---3' (SEQ ID NO: 166)

Overlapping with UDEC1713 (24bp)

UDEC1713 3' primer 239 bp

5'---CTTTCGCTTCGCGCGGATAAAAGTTGTTTCAGCAGGCACACCACGCTCGCGGTGCCGCTTTTCAGTGTTCA

Overlapping with UDEC1714

TCGCTCGGCGGAAAAATAAACACGCTCGGCGCCGCCACGGTGCGTTTAATTTCCACTTTGGTGCCCTGGCCAAAGGT
CCACGGGCTGCTGCCATACTGCTGGCAATAATACACCGCAAATCTTCCGGTTCCAGGCGGCTAATGGTCAGGGTAA
AATCGGTGCCGCTG---3' (SEQ ID NO: 167)

Overlapping with UDEC1712 (24bp)

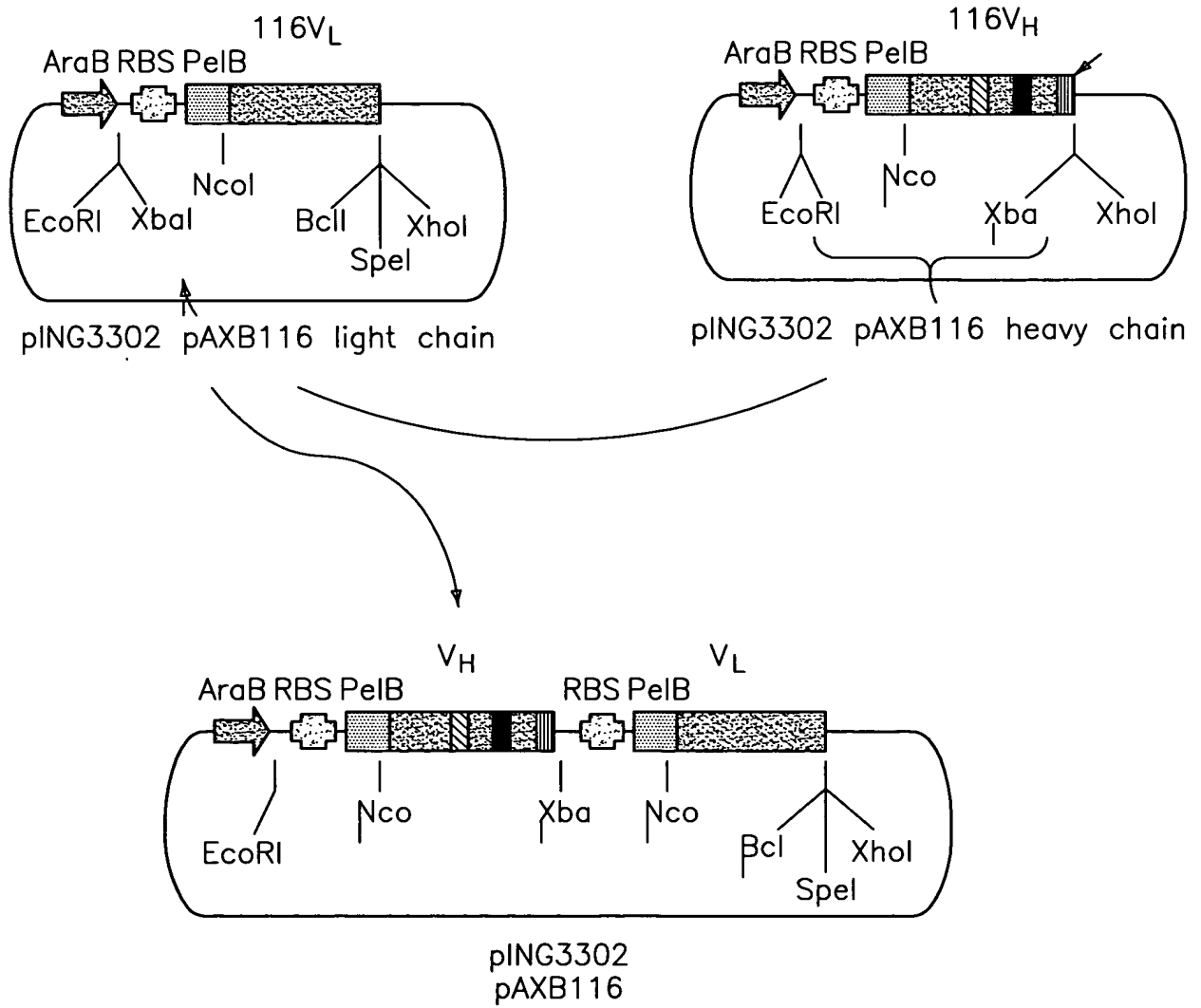
UDEC1714 3' primer 245 bp

5'----GTGCTGATCATTAGCATTCGCCGCGGTTAAAGCTTTTGGTCACCGGCAGGCTCAGGCCCTGATGGGTCACTTC
GCACGCATACACTTTATGTTTTTTCATAATCCGCTTTGCTCAGGGTCAGGGTGCTGCTCAGGCTATAGGTGCTATCTTT
GCTATCCTGTTTCGGTCACGCTTTTCTGGCTGTTGCCGCTCTGCAGCGCGTTATCCACTTTCCACTGCACCTTCGCTTCG
CGCGGATAAAAGTTG---3' (SEQ ID NO: 168)

Overlapping with UDEC1713 (26bp)

FIG. 27

Construction scheme for pING-pAXB116

**FIG. 28**

116 Light Chain (SEQ. ID NO. 122):

EIVLTQSPGTL^{SL}SPGERATL^{SC}RASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSLPVTKSFNRGEG.

Variable Region of 116 Light Chain (SEQ. ID NO. 123):

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDEAVYYCQQYGSSPWTEGGGTKVEIK

116 Heavy Chain (SEQ. ID NO. 124):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVITADESTSTAYMELSSLRSEDTAVYYCARLP IEGPTLRQWLAARAPVWGQGT VTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK {VEPKSCDKTHTCPPCP} APELLGGP
end CH1 constant domain hinge region tail region

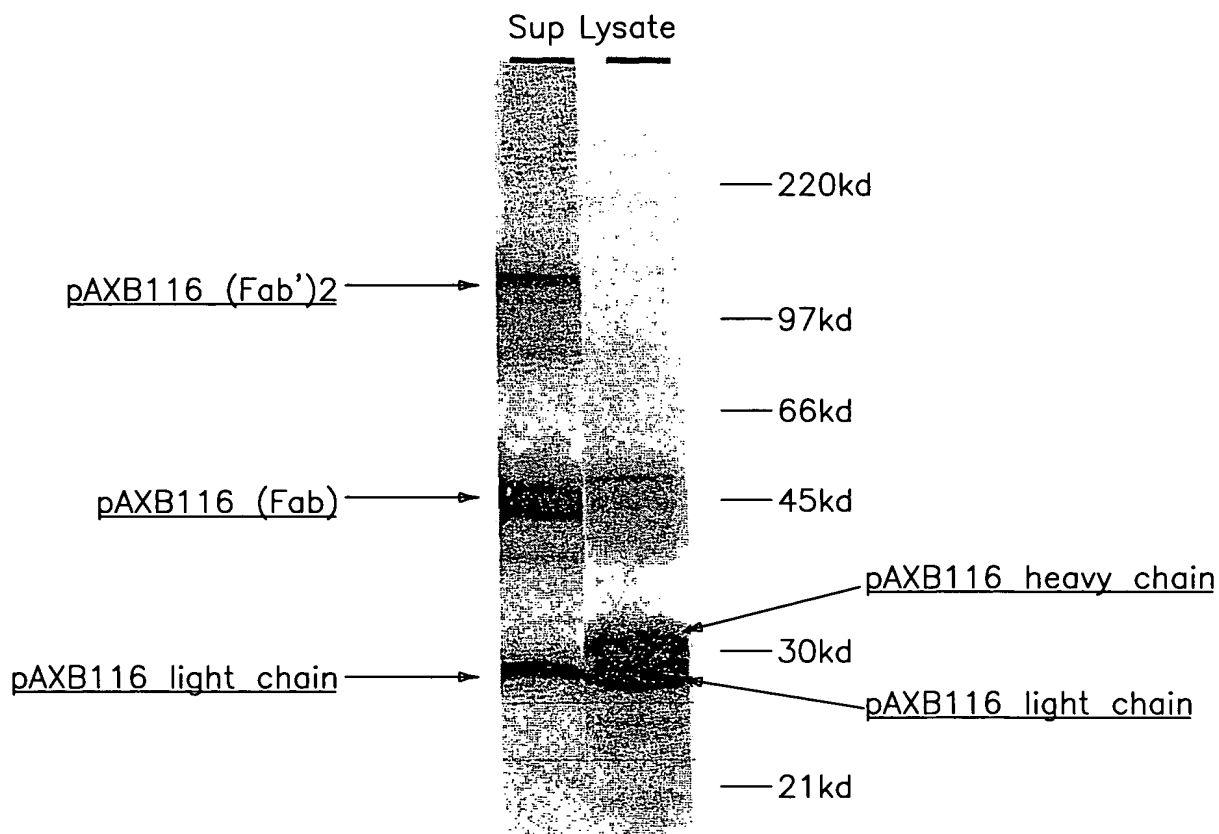
Variable Region of 116 Heavy Chain (SEQ. ID NO. 125):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSED TAVYYCARLP IEGPTLRQWLAARAPVWGQGT TVTV
SS

Clone 116. The light chain sequence is as given. The heavy chain may have several forms depending on the final antibody form. For example: the minimum sequence ending with the CH1 constant domain may result in Fab assembly but the heavy chain/light chain interaction will not be stable. Commonly, a portion of the hinge region containing a cysteine (the underlined bold portion of hinge region) may be included for a covalent interaction between the heavy and light chains. Fab'₂ association would need further cysteines such as an entire IgG₁ hinge region (bold). In this example, clone 116 was cloned in a Xoma pING3302 modified vector which includes a transition tail region (italicized).

FIG. 29

SDS-PAGE of pAXB116, culture supernatant was resolved from each other by non-reducing 4-12% SDS-PAGE (lane "Sup") and cell lysate by reducing 4-12% SDS-PAGE (lane "Lysate"). Proteins were transferred onto Hybond Nitrocellulose Sheet (Amersham) and block with TBS-0.2% Tween-20+10% (w/v) Carnation nonfat dry milk. PAXb116 were detected by HRP-conjugated goat-antiHuman (H+L)Ab (Chenicon Cat#AP112P, (Temecula, CA). The signals were detected by ECL.



WB:Goat anti human(H+L) HRP-!:1000

FIG. 30

CD34⁺ cord blood cells (Poeisis) were thawed, washed, resuspended in BIT9500 serum-substituted medium (StemCell Technologies, Inc.), and plated at 3.5×10^5 per well in a 96 well flat-bottom plate with increasing concentrations of either recombinant human TPO (R&D Systems), circle, or pAXB116, square. After four days of culture at 37°C in a 5% CO₂ incubator, 1 Ci of ³H thymidine (Perkin Elmer) was added to each well and cells were further incubated for 16 hours. Cells were harvested with an automatic 96-well cell harvester. ³H incorporation was measured using a betaplate liquid scintillation counter (Wallac). Proliferation of cord blood cells are measured as counts per minute (CPM) and CPM values are an average of three wells.

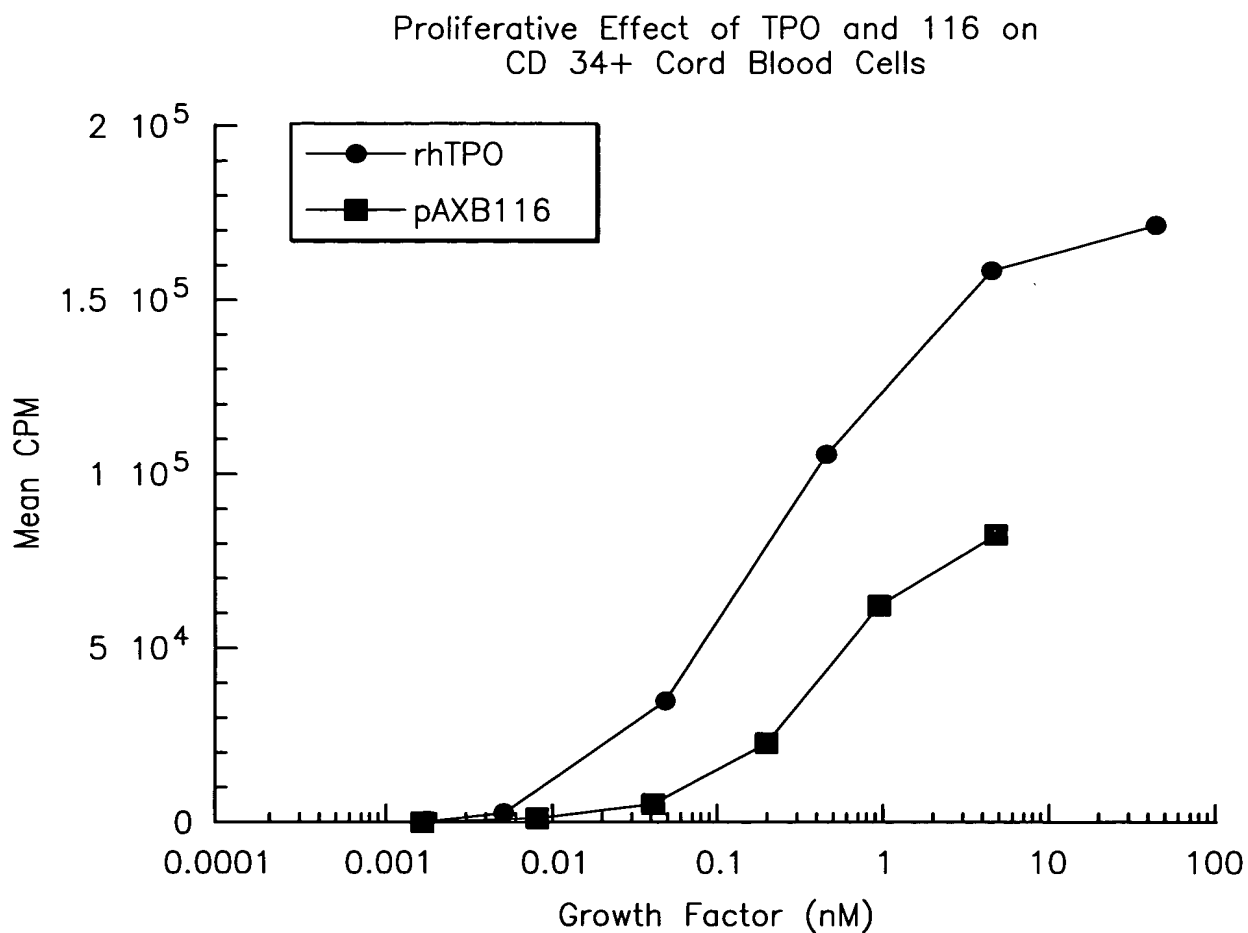
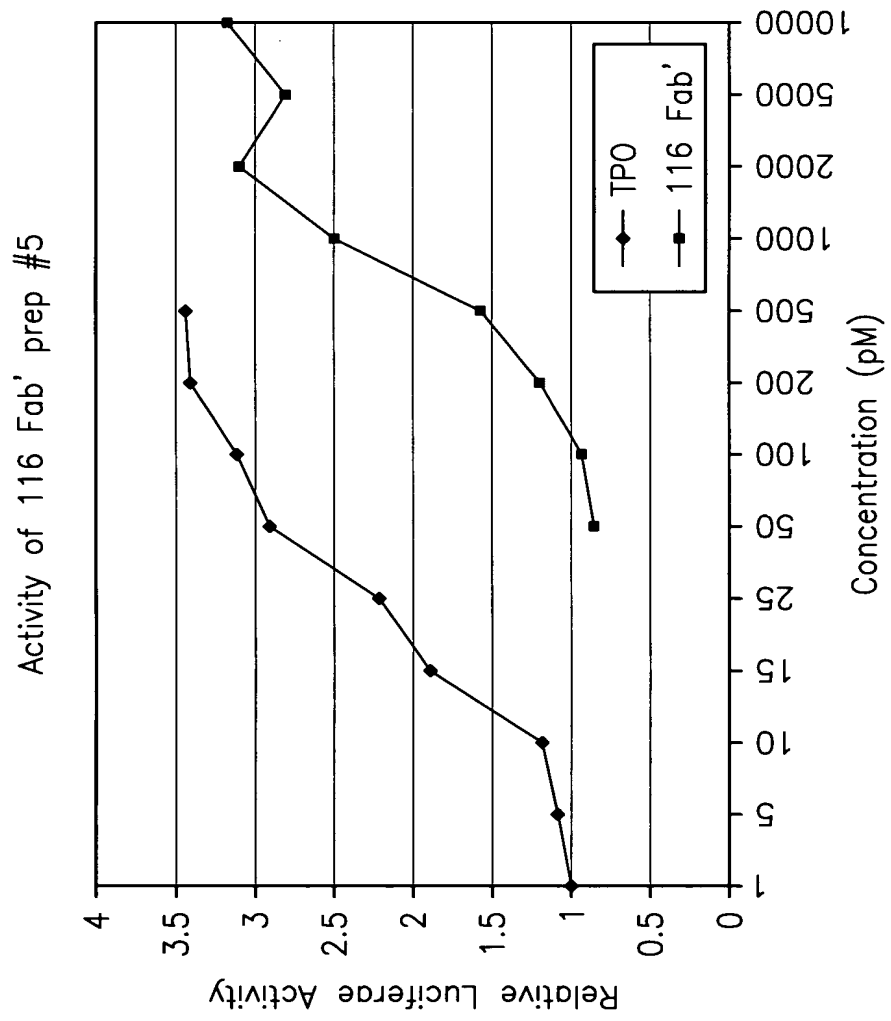


FIG. 31

*FIG. 32*

Sample Sequences of Heavy Chain CDR2 clones

<u>Gly-Ile-Phe-xxx-xxx-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-xxx-xxx-Gly</u> (SEQ. ID NO. 126)				
TT backbone	randomized	TP0 peptide	randomized	TT backbone
Amino Acid Sequence				
HR2-14	<u>Gly-Ile-Phe-Ser-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Ala-Gly-Gly</u> (SEQ. ID NO. 127)			
HR2-20	<u>Gly-Ile-Phe-Pro-Gln-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-His-Gly</u> (SEQ. ID NO. 128)			
HR2-23	<u>Gly-Ile-Phe-Pro-Asn-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Thr-Gly-Gly</u> (SEQ. ID NO. 129)			
HR2-28	<u>Gly-Ile-Phe-Lys-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Gly-Gly</u> (SEQ. ID NO. 130)			
HR2-43	<u>Gly-Ile-Phe-Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Ala-Val-Gly</u> (SEQ. ID NO. 131)			
HR2-44	<u>Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Leu-Gly</u> (SEQ. ID NO. 132)			
HR2-48	<u>Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Leu-Gly</u> (SEQ. ID NO. 133)			
HR2-50	<u>Gly-Ile-Phe-Pro-Tyr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Arg-Gly</u> (SEQ. ID NO. 134)			

FIG. 33

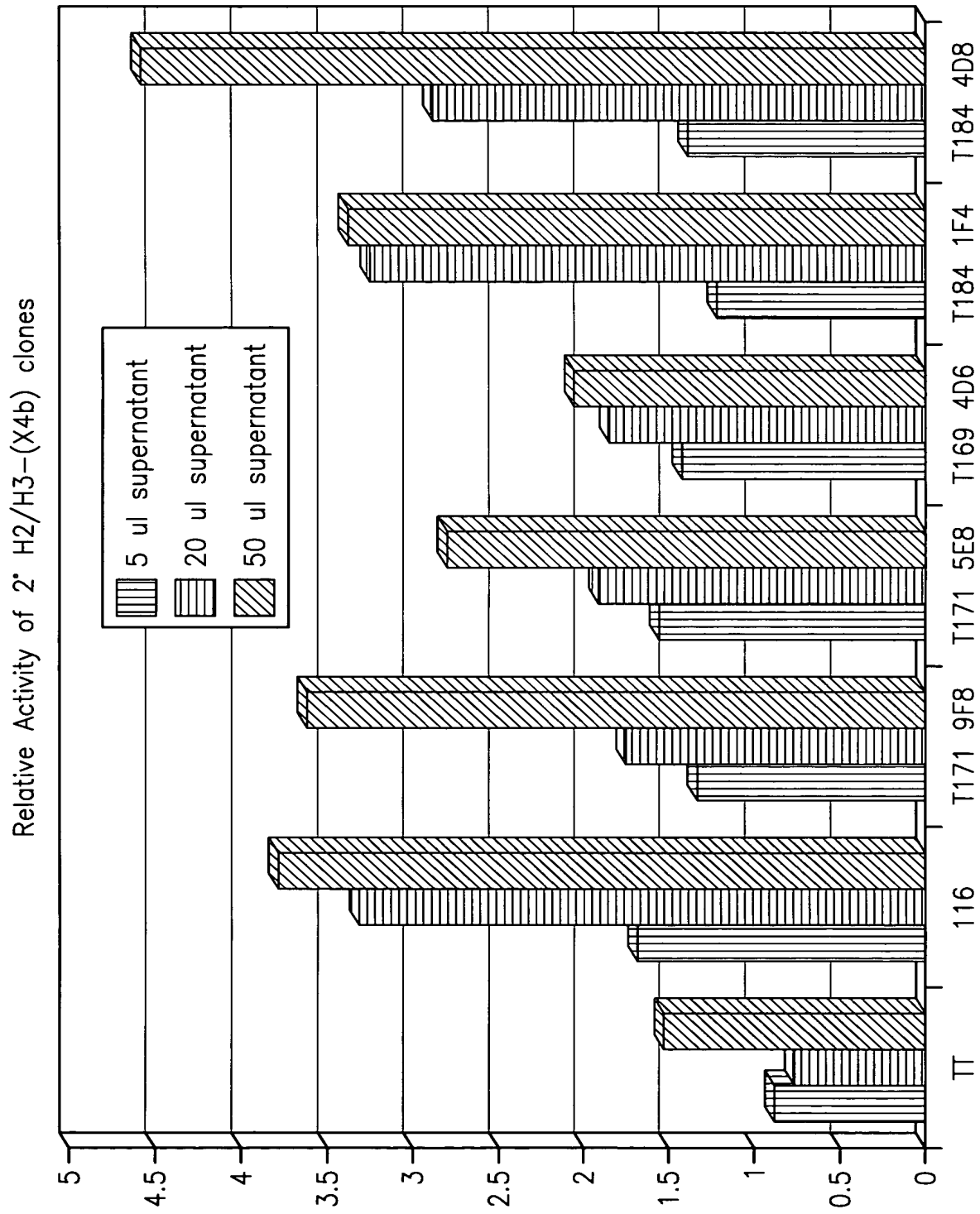


FIG. 34

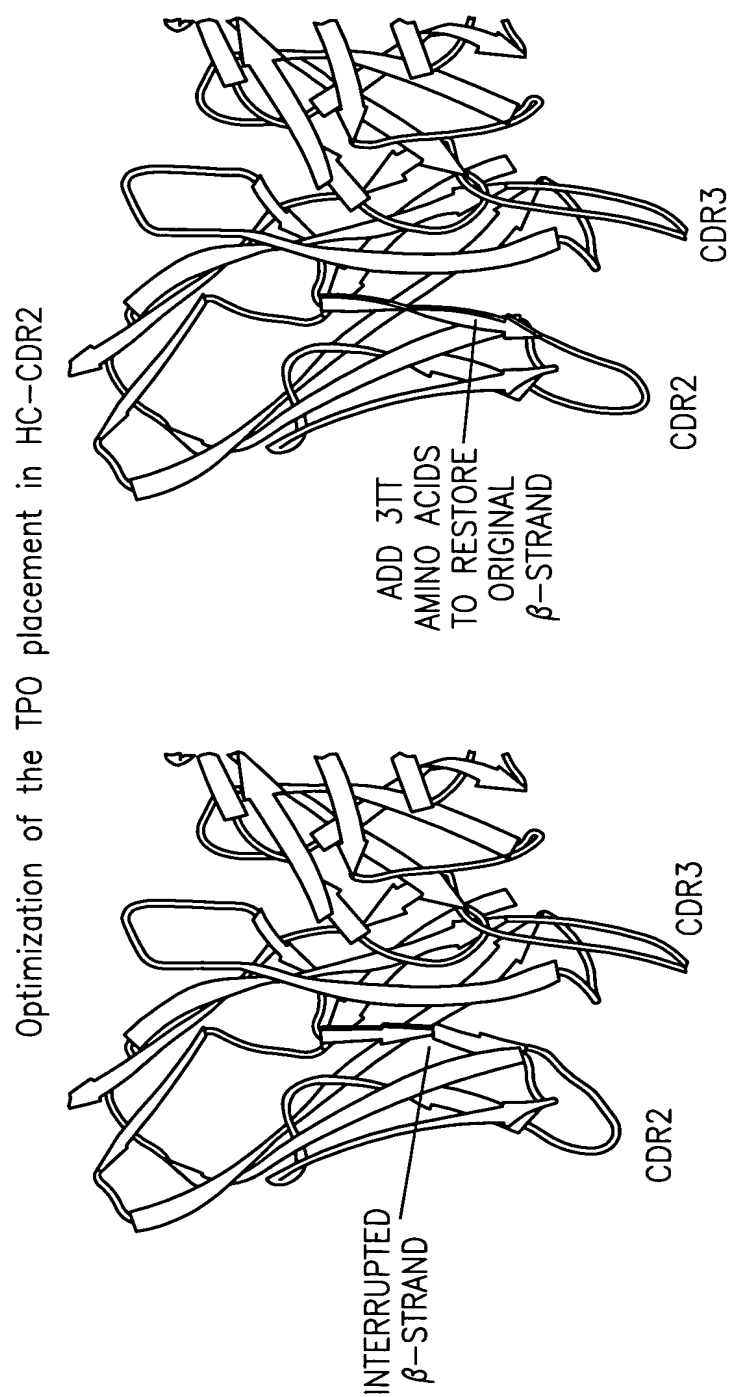


FIG. 35

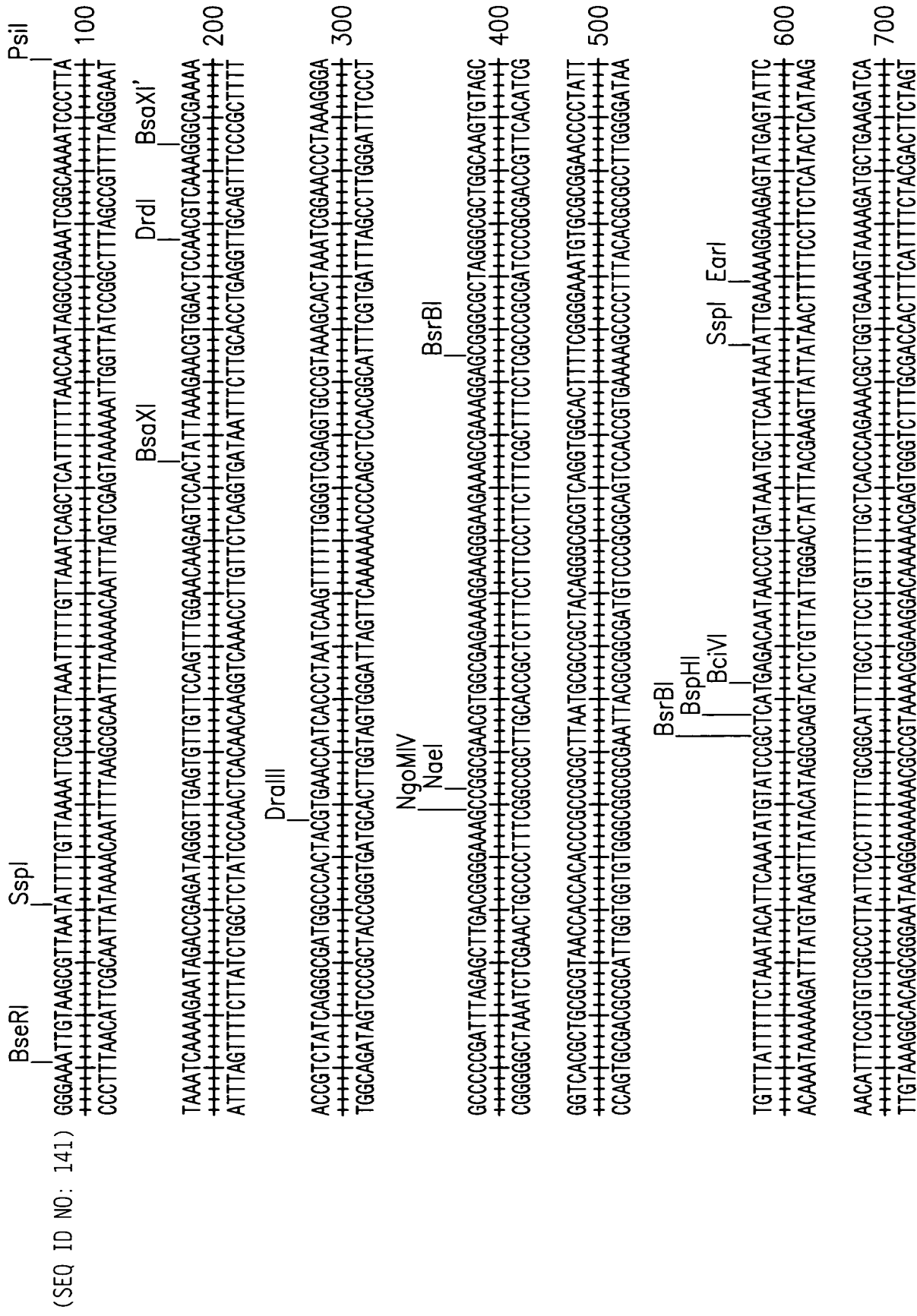


FIG. 36A

FIG. 36C



FIG. 36D

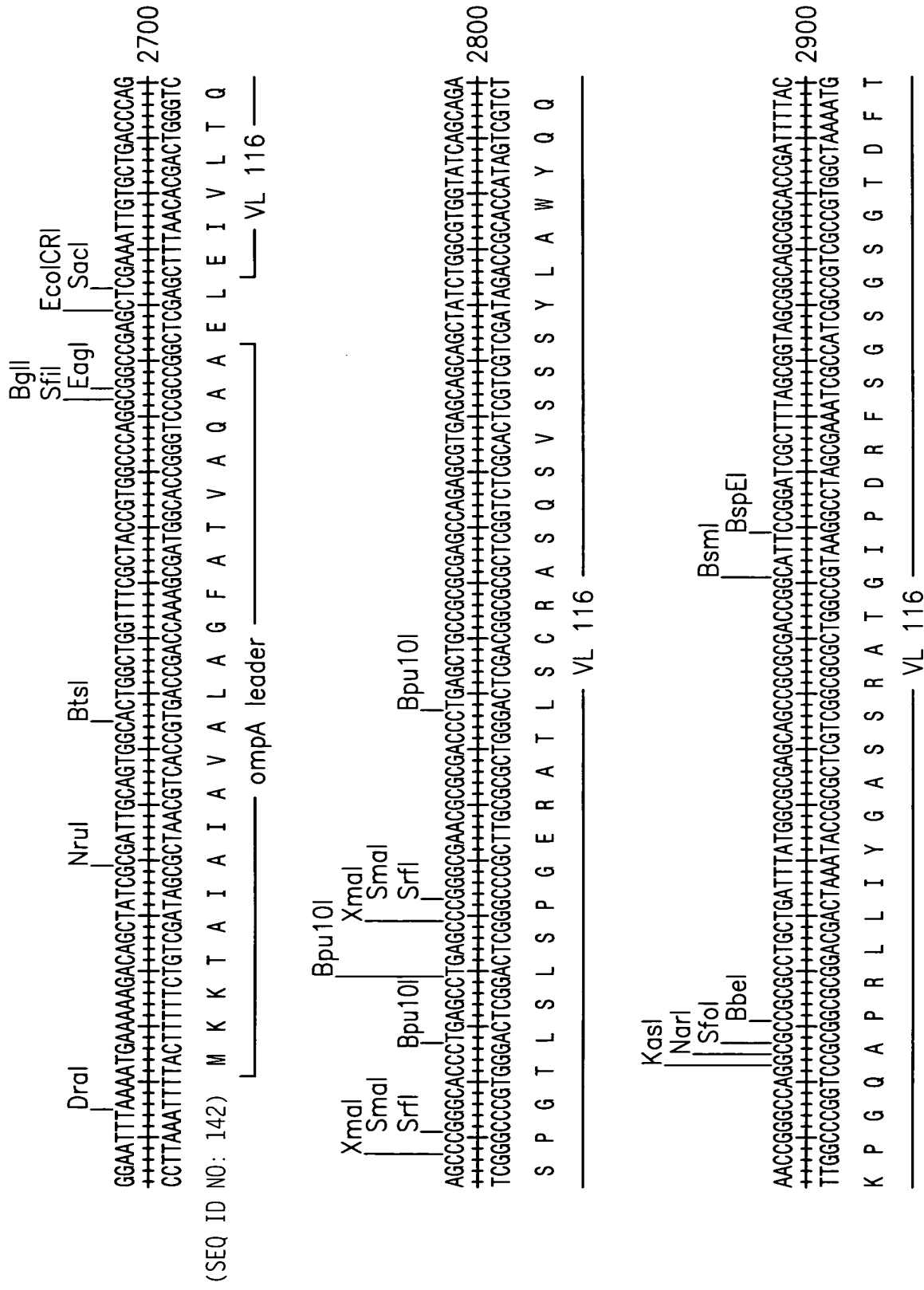


FIG. 36E

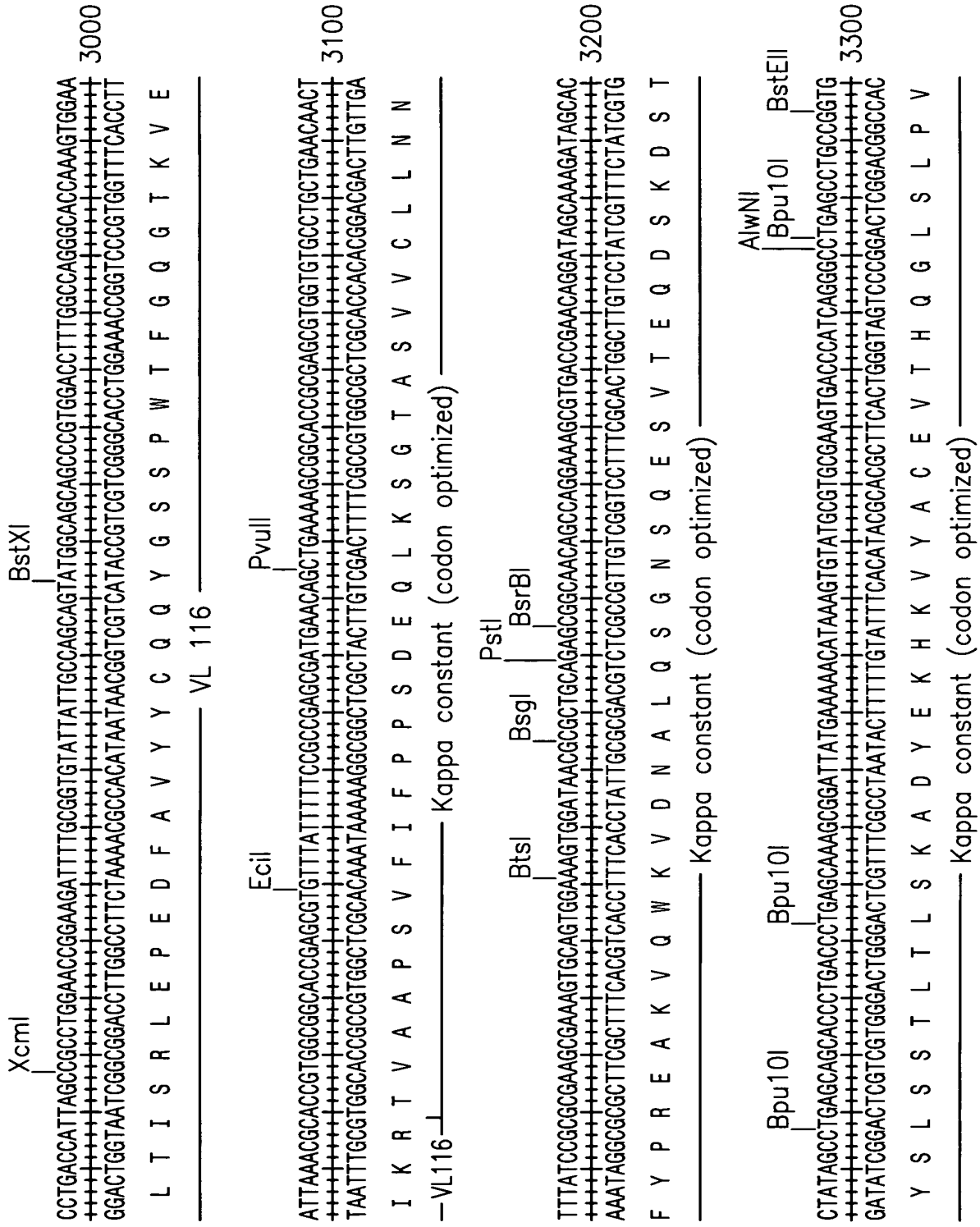


FIG. 36F

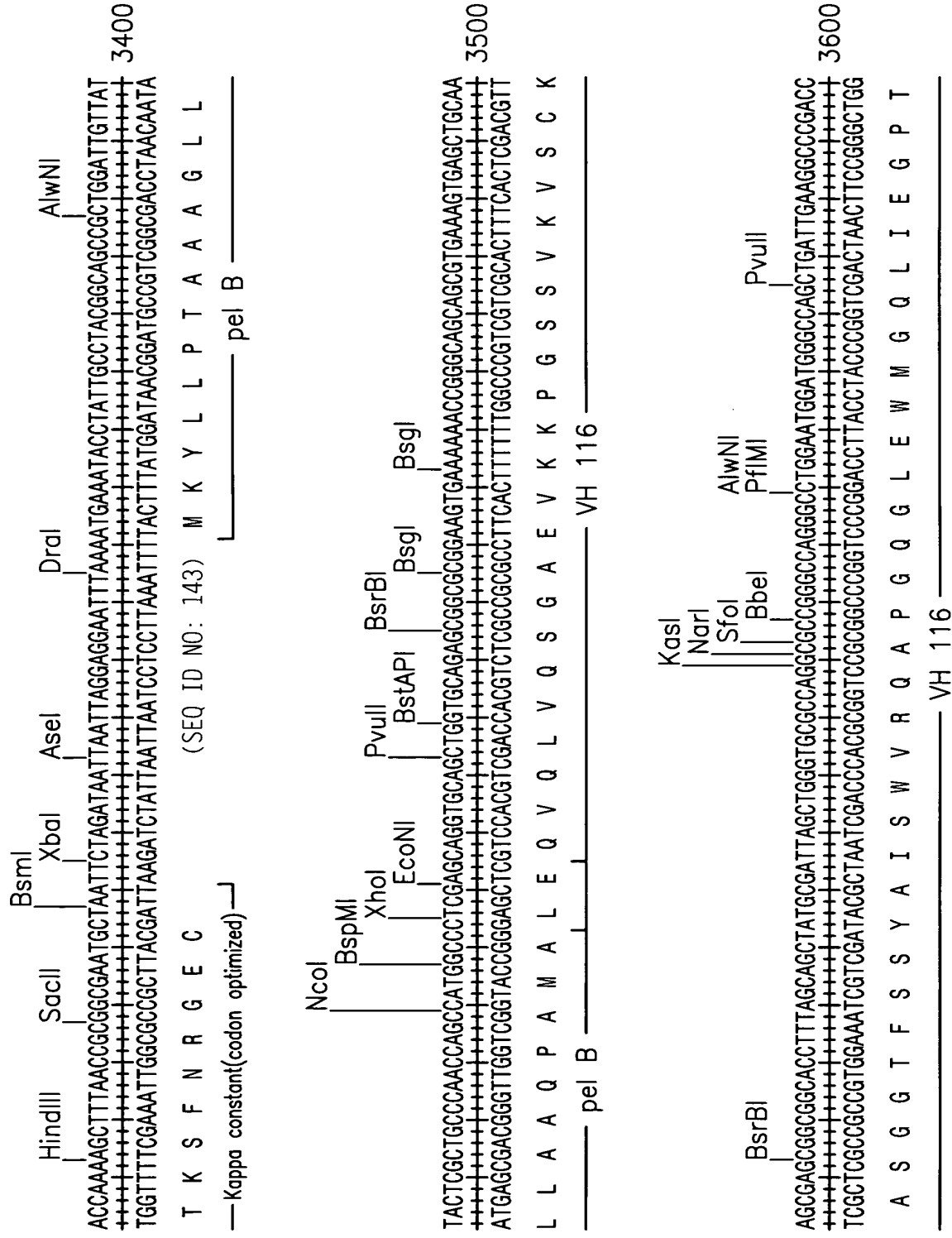


FIG. 36G

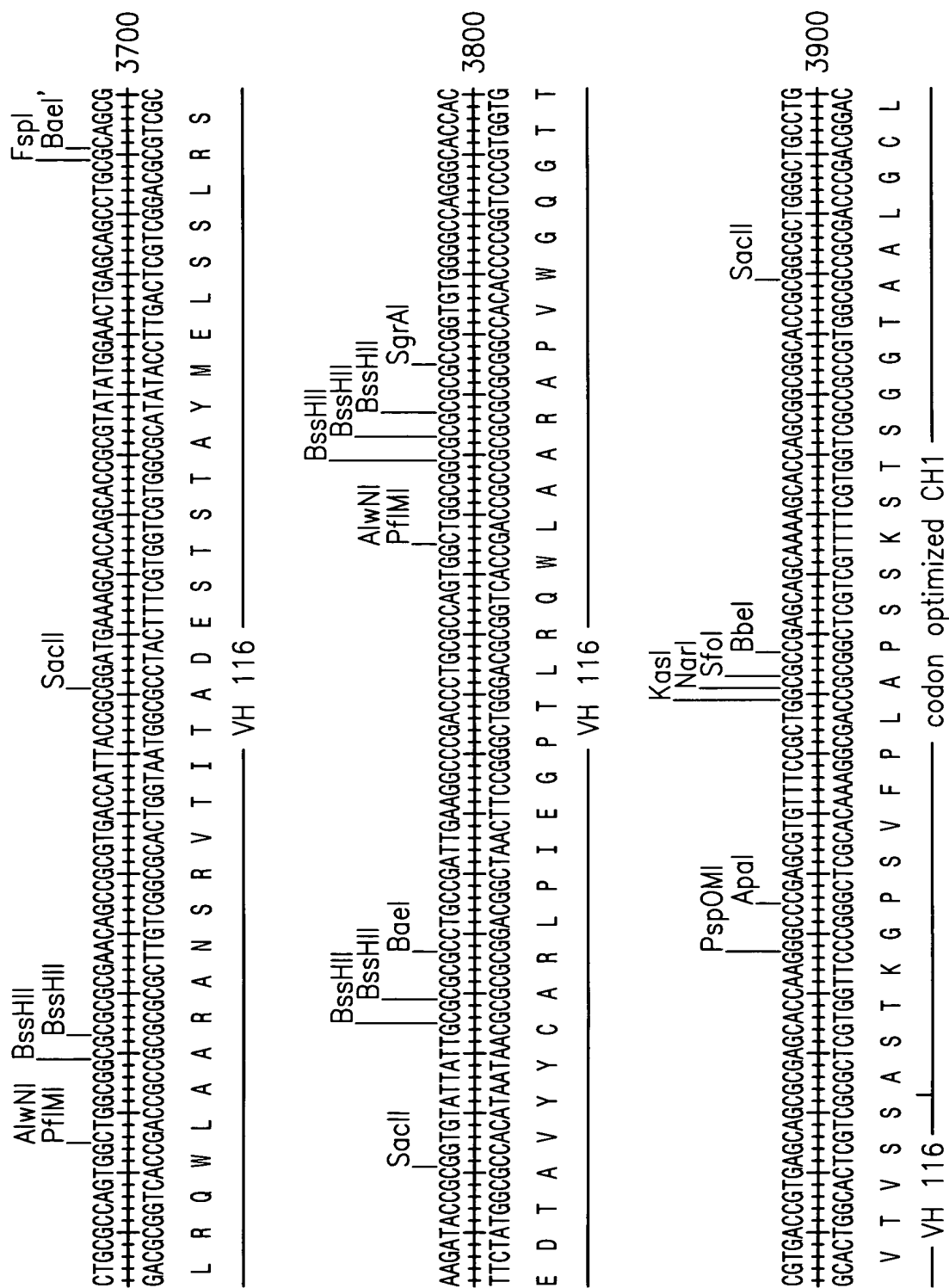


FIG. 36H

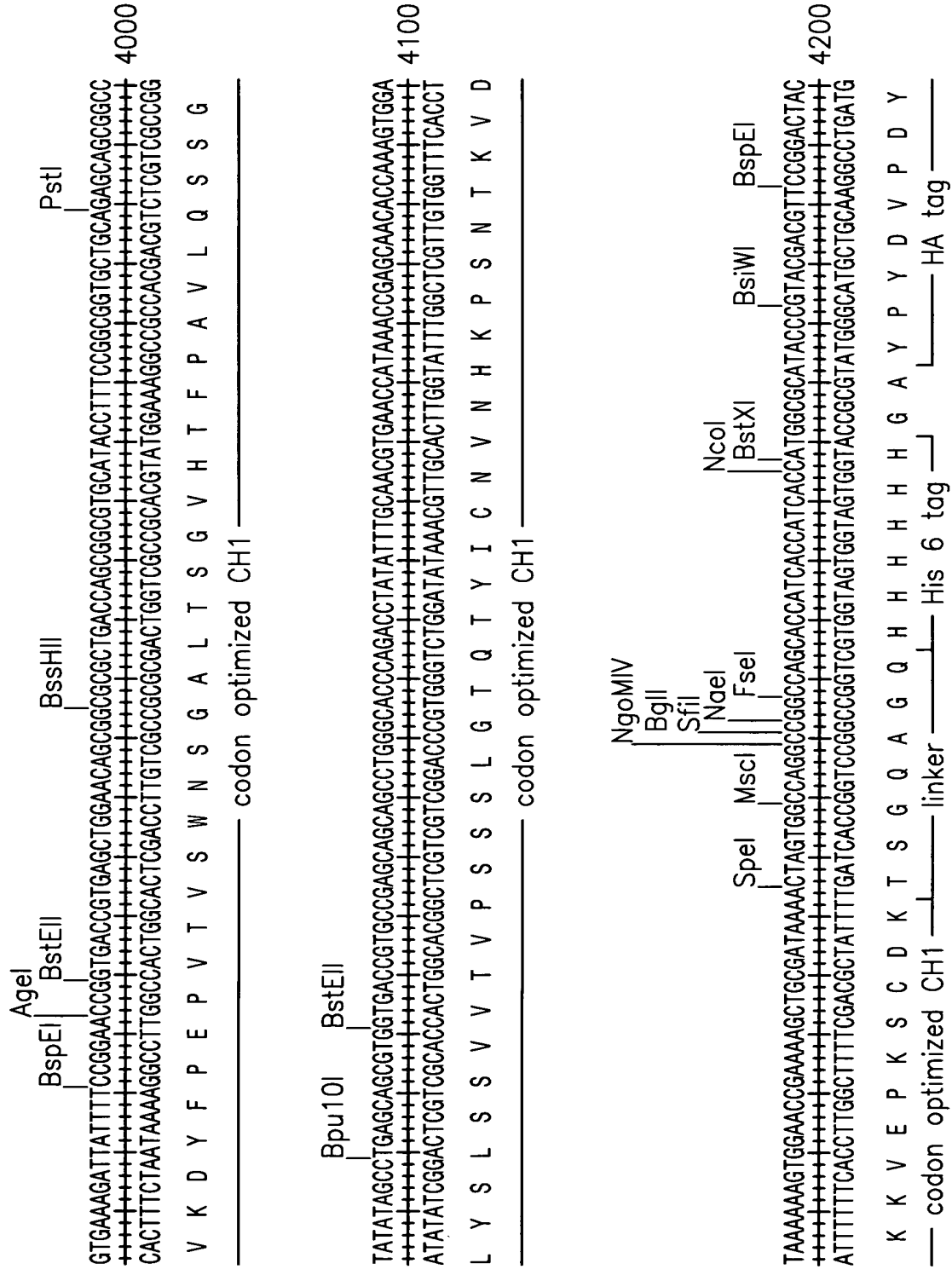


FIG. 36I

4300

gene III fragment

(SEQ. ID NO. 171)

4400

gene III fragment

 $\frac{C}{d}$

4500

gene III fragment

BsaXI
XmnI | SspI
BsaXI'

gene III fragment

FIG. 36J

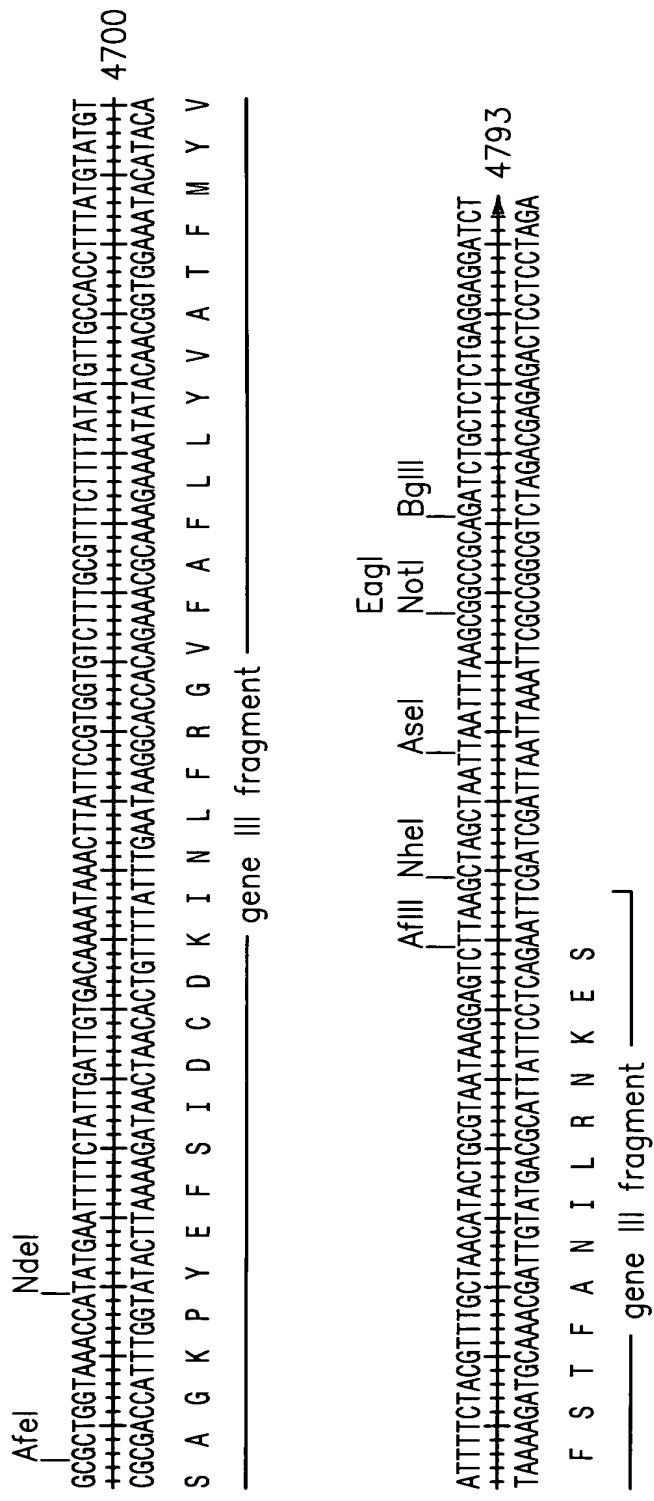
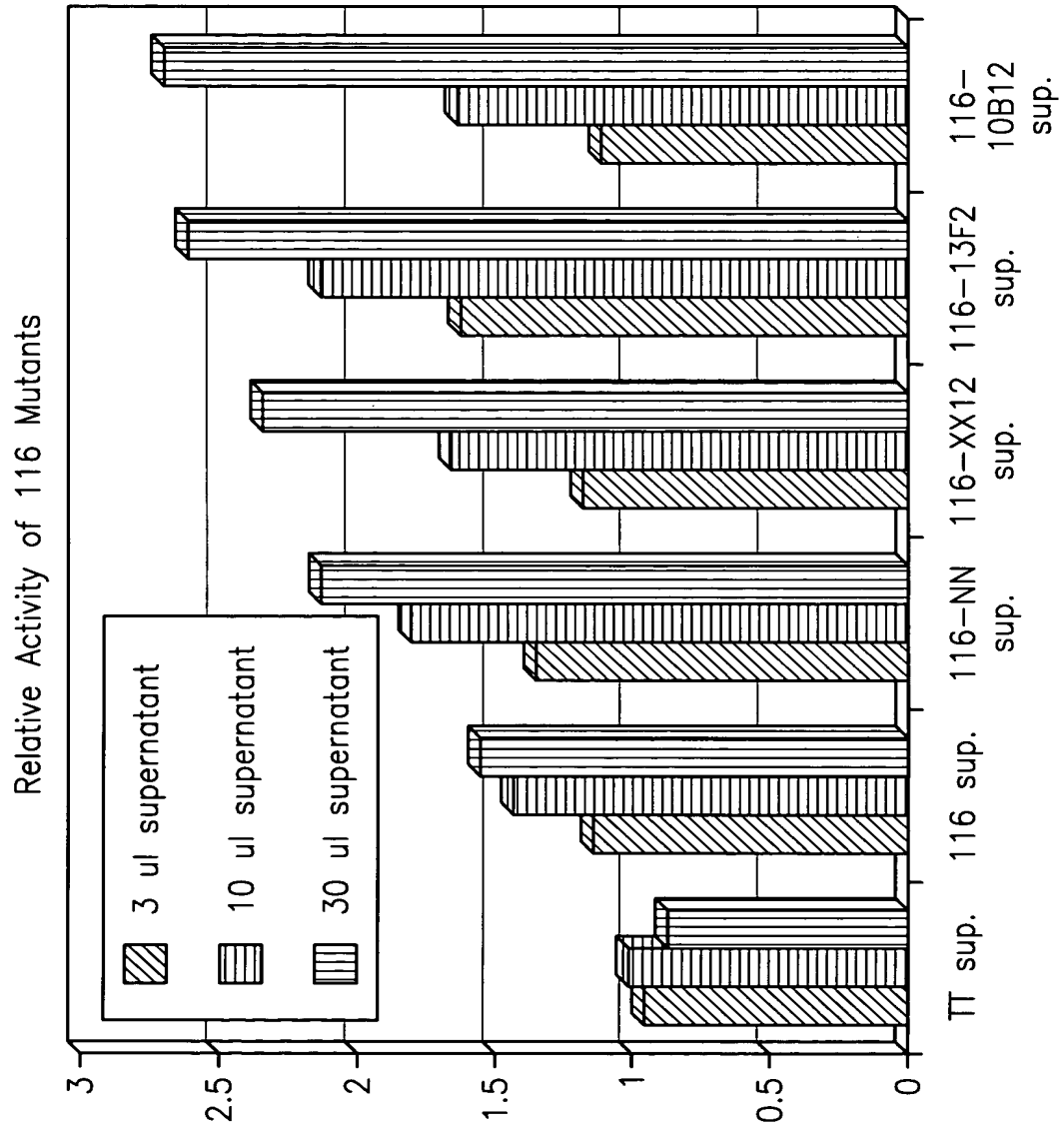


FIG. 36K

*FIG. 37*

	10	20	30	
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S			pRL5-116 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F N			pRL5-116 NN (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F G			pRL5-116 10B12 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F Q			pRL5-116 13F2 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F P			pRL5-116 XX12 (VH)
	40	50	60	
31	S Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 (VH)
31	N Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 NN (VH)
31	E Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 10B12 (VH)
31	D Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 13F2 (VH)
31	R Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 XX12 (VH)
	70	80	90	
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 NN (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 10B12 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 13F2 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 XX12 (VH)
	100	110	120	
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 NN (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 10B12 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 13F2 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 XX12 (VH)
121	G T T V T V S S	(SEQ. ID NO. 147)		pRL5-116 (VH)
121	G T T V T V S S	(SEQ. ID NO. 148)		pRL5-116 NN (VH)
121	G T T V T V S S	(SEQ. ID NO. 149)		pRL5-116 10B12 (VH)
121	G T T V T V S S	(SEQ. ID NO. 150)		pRL5-116 13F2 (VH)
121	G T T V T V S S	(SEQ. ID NO. 151)		pRL5-116 XX12 (VH)

116 Variants Alignment

FIG. 38